Plant Developmental Biology
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Lecture - 16
Shoot Development: Organogenesis

Welcome back to Plant Developmental Biology course. So, we are studying today Shoot Development particularly Organogenesis part.

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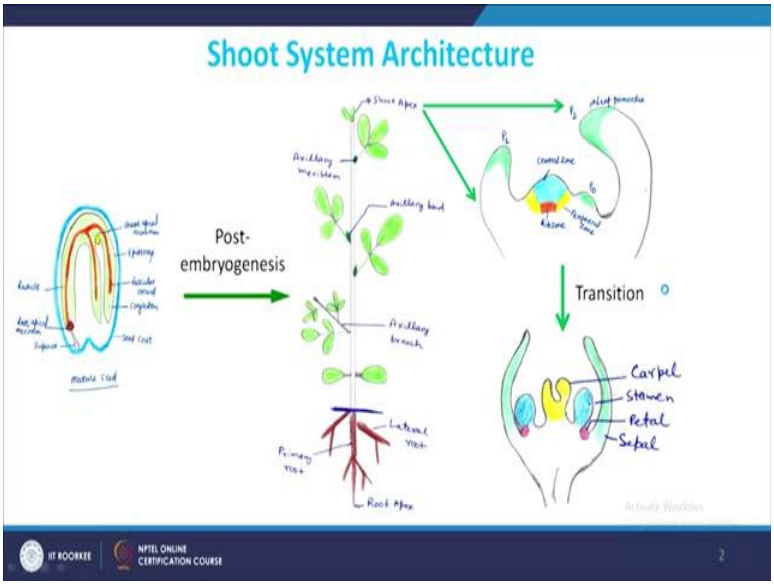

In the last class we have studied shoot apical meristem or maintenance and here in this class we are going to study organogenesis. This is typical shoot system in plant and in shoot system we have organs like leaves and axillary branches and at the apex we have shoot apical meristem during vegetative phase. But, when the transition occurs from vegetative phase to reproductive phase, this shoot apex or shoot apical meristem get converted into inflorescence meristem and then inflorescence meristem makes the flower. So, these are the typical part of shoot system.

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And you have seen in previous classes that how shoot apical meristems are organized during vegetative growth and development. This is a vegetative shoot apical meristem and if you remember there is a central zone, rib zone and peripheral zone, and this is the top view of a growing shoot apical meristem. So, the central region is shoot apical meristem, and the peripheral zone or peripheral region of the shoot apical meristem, these are the organs which are under the process of development.

During the growth and development the meristematic activity is maintained in the very tip of the meristem. When the cells which are produced in the meristem enters in the peripheral zones they basically initiate the process of differentiation.

Here is the view of inflorescence meristem, it looks very similar. In inflorescence meristem also you have a central zone then you have peripheral zones and rib zones and stem cells are maintained in the central zone. This is the region where cell division activity is very dominant and differentiation is inhibited. But when this cells enters in the peripheral zones they basically initiate organ specific developmental program depending on the fact whether if they are in the vegetative phase, the lateral organs are going to be the leaf if they are in the reproductive phase, the lateral organs are going to be a flower.

Here both the process of meristem maintenance and the process of organogenesis is highly coordinated and it occur simultaneously. If this does not happens, if less assume that meristematic activity is more than the differentiation activity then the meristem size will keep on increasing.

On the other hand if you have more differentiation activity then meristems will be consumed early; under both the conditions you are going to see abnormal growth and development. So, during the process; both the process has to be coordinating. In last class we have seen how meristems are getting regulated, what are the genetic regulatory pathways particularly *WUSCHEL* and *CLAVATA* mediated pathway and how they basically ensures meristematic activity in the meristematic zone.

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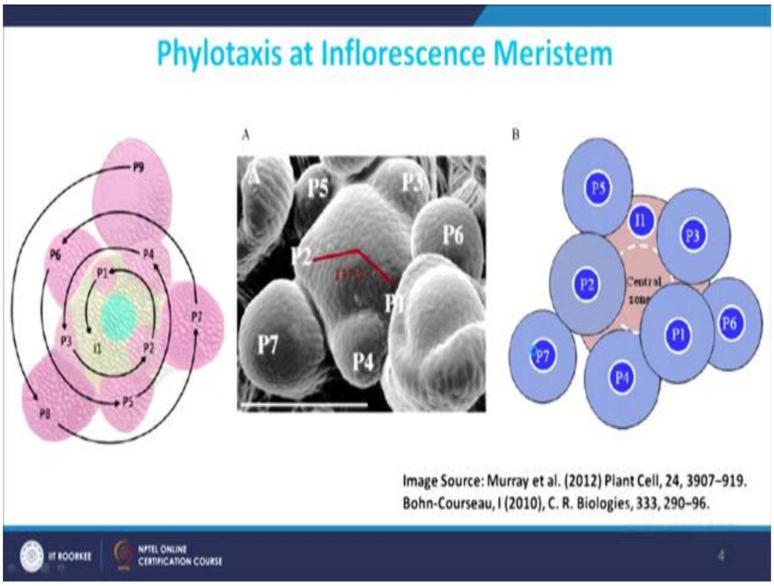

Here in this class we are going to see how the differentiations are initiated.

So, what happens in the differentiation, if you look this is a central meristem and in the peripheral meristem, first side where the process initiate is called I1, I2 like that and then you have a leaf primordia like P1, P2, P3, P4, P5, P6 and so on. There is a clear defined pattern of organogenesis. Organogenesis is not a very random process, if you look the top view of a growing meristem that between P1 and P2 there is a typical angle. SThe positioning the primordia is another very important and critical parameter during the process of organogenesis.

Everything is being maintained here and how these primordia's are positioned, how it is being define then there was a hypothesis that some kind of inhibitory zones are created around a primordia. So, if you look if these are the primordia, the circles basically denotes the inhibitory zones, which means that in this region or around this primordia up to this region there is no other primordia will originates, but question is still here that how and so precisely this inhibitory domains are maintained.

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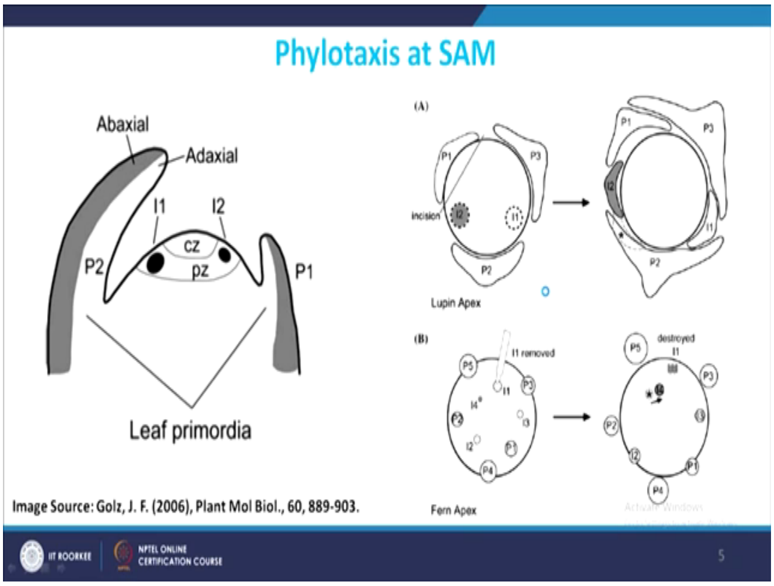

A coordination and communication between meristem and growing primordia is very important. How will you prove this?

For example, if you create a kind of incision between growing primordia and the meristem, if you physically disturb or you are removing one of the developing primordia, what you are getting here that the patterning or phylotaxis is disturbed. For example, if you make incision between P1 and this center of a meristem what you see that the positioning of I2 is now changed. So, it is basically shifted from the original position.

Similarly, if you remove or somehow kill one of the primordia, there is a migration or there is a change in the position of ah other primordia. So, this tells that some kind of communication between meristem and the primordia is going on and this is absolutely important for positioning primordia in the growing meristem. What could be that signal or what could be that communication way?

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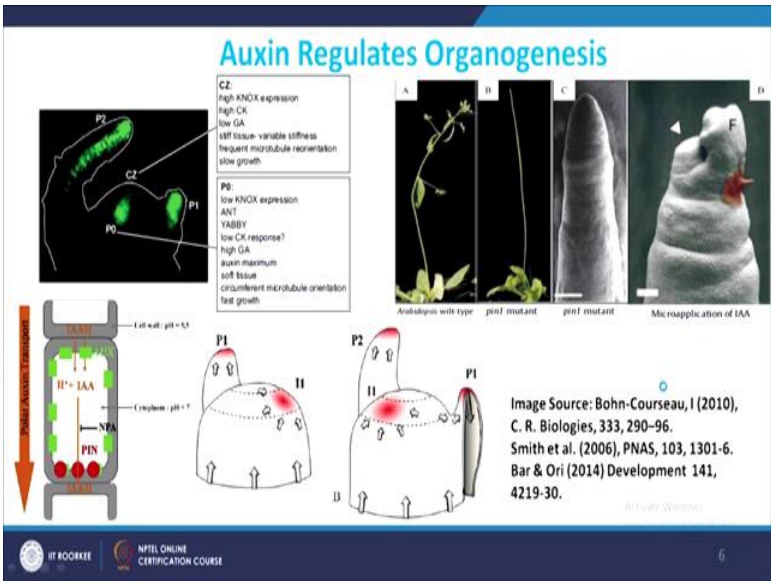

Auxin hormone which functions like a morphogen is absolutely important for entire growth and development. And if you look the DR5 activity which tells the auxin response activity, in the growing primordia auxin amount is very high. In some of the mutants such as *pin* mutant; PIN is basically a protein which is responsible for polar transport of the auxin. The *pin* mutants, if you do not have PIN protein which means that the auxin is not getting distributed properly you look the structure that organogenesis is almost blocked and the structure looks like a pin. Whereas, if you just apply little bit of auxin exogenously, micro application of auxin you can see that organogenesis can initiates.

So, this suggest that auxin is playing very important role in the organogenesis or in the primordia differentiation. How the auxin basically works? So, auxin is bio synthesized in some of the cells and then they can be distributed or they can be transported and the transport of auxins occurs through a well established auxin transport system which is also called polar auxin transport system. Because the transport of auxin is a directional. IAA is one of the auxin in the cell. There are two kinds of protein AUX protein and PIN protein.

PIN proteins are auxin efflux carrier AUX protein are auxin influx carrier. Through these transport systems auxins are being transported from one cell to another cells. Another very important thing which is always associated with the PIN proteins are that they can be asymmetrically localized in the cell. One particular cell wall may have the PIN proteins, which means that they are asymmetric localization in the cell wall can provide a direction for auxin movement.

If PIN is localized here then auxin can move from here to here, and this kind of polar auxin transport basically helps in gaining auxin maxima in some of the tissues. So, one hypothesis was that there could be that auxin is getting accumulated very high in the primordia and this is happening through the polar auxin transport. And this has been tested and this has been shown in a great detail using different mutants or different approaches.

If this is a meristem, then auxins are getting transported towards the position where a primordia has to be initiated. And this is how the region or the tissues they basically achieve auxin maxima, and when auxin level is increased beyond a certain point it initiate a primordia specific developmental program, then the auxin can be diverted from that place, so that a crucial balance or homeostasis of auxin is maintained.

If you look the central zone, in central zone cytokinin is predominant and then you have *KNOX* gene which is responsible for the maintenance, but in the primordia *KNOX* gene expression is low cytokinin is low, but auxin is high. So, the critical balance between auxin and cytokinin and some of the key transcription factors defines the meristematic activity in the meristem and differentiation activity in the primordia.

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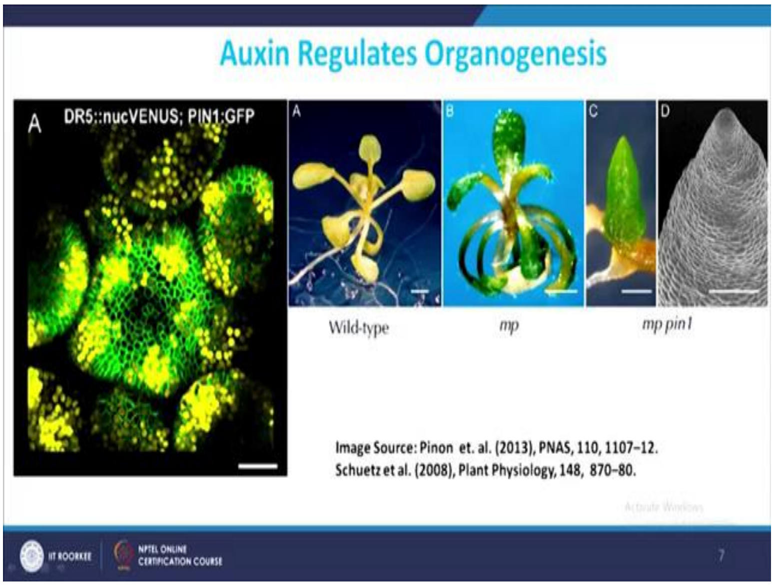

This you can also see by the genetic interaction. This is a double marker; here you have DR5, a auxin responsive promoter driving a nuclear localized yellow signal which is basically auxin response and *PIN* protein has a GFP. So, you can see the GFP localization as well as the auxin response and you can see that auxin response are very high in the primordia.

And MP is a transcription factor which is an auxin response transcription factor and MP is regulated by auxin and what happens if you have single mutant of *mp* you can see that the differentiation or the phyllotaxis or the organogenesis is disturbed. But if you combined *mp* with the *pin* mutants, you have the auxin response factor mutant as well as the polar auxin transport mutants, so you can see the phenotype where organogenesis is almost blocked.

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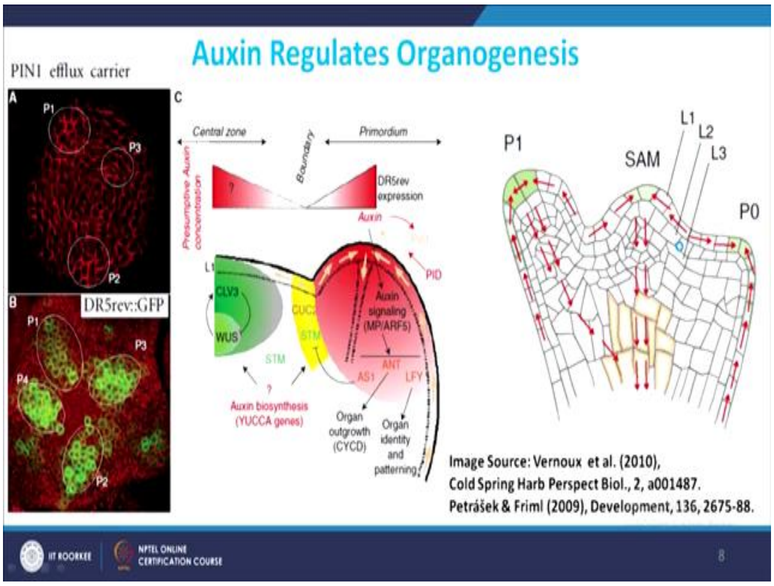

If I summarize here you have auxin and then auxin is getting diverted in the site of primordia through the polar auxin transport. And then in the primordia you are going to have a very high amount of auxin and this auxin initiate the program and then auxin has been diverted from there through the vascular tissues.

So, this is what happens in the growing meristem. You have a central meristematic zone and you have a peripheral primordia and also the border or the boundary between meristem and primordia this has to be defined.

So, up to what region the cell division activity should occur and then when the differentiation initiate and this kind of boundaries are maintained by some of the genes which are very specifically expressed in the in the boundary for example, *CUC2* they are NAC domain containing and some of them are LOB; LOB is lateral organ boundary domain containing transcription factor.

So, if you look here in the meristem you have *WUSCHEL* and *CLAVATA* activity. High cytokinin, *WUSCHEL* and *CLAVATA* activity and the meristem regulating gene like *STM* they ensures meristematic activity here, but if you look in the primordia you have high amount of auxin. Auxin is activating, *AUXIN RESPONSE FACTOR5* or *MP* and then they are activating some leaf primordia or floral primordia a specific genes which are basically regulating primordia differentiation.

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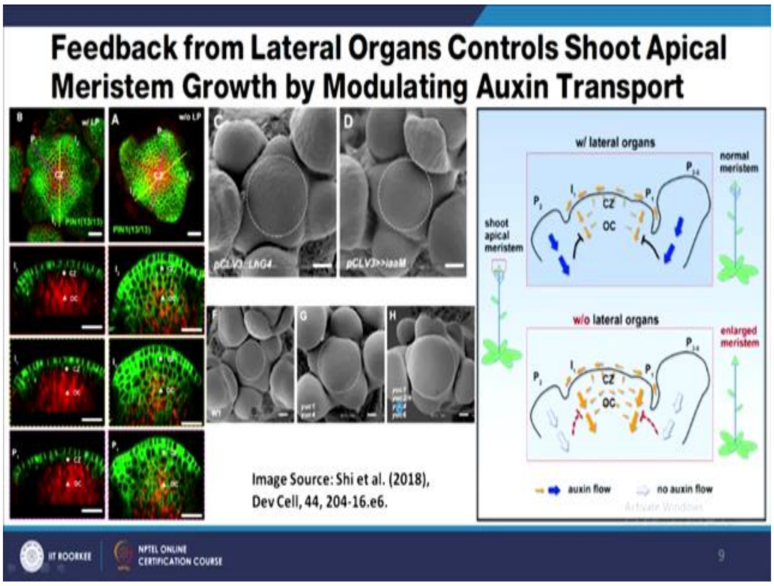

The coordination between meristem and the lateral organs is well established. There is a feedback from lateral organs which also controls the shoot apical meristem activity. So, it is signaling both way. Meristem is controlling the activity of lateral organ growth and lateral organ is going to control the meristematic activity, how?

If you look here PIN protein localization in the wild type; this is primordia P1, I1, I2, if you look the PIN protein; PIN protein is mostly localize to the L1 layer. This is a normal growing primordia, but if you remove one lateral primordia which could be leaf primordia if you just remove it what is happening that distribution or localization of PIN protein is totally disturbed here.

So, this tells that the presence of lateral organ or development of lateral organ in the peripheral zone is sending some kind of signal to the meristem for its own activity. And if you produce auxin in high amount so, basically in the *CLAVATA3* promoter *CLAVATA3* is expressed in the central region and if you synthesize auxin what you see that the meristematic zone is decreased, the meristem size is decreased. On the other hand if you remove auxin from the meristem, this *YUCCA* genes are auxin biosynthesis genes.

So, if you have double mutant of *YUCCA1* and *4* or triple or four mutants of different members of *YUCCA* gene family. So, essentially what you are doing? You are reducing total bio synthesis of auxin and in that case what you can see that the meristem size is increasing.

So, this tells that there is a clear feedback signaling between lateral organs as well as the meristem. So, under normal condition when your shoot apex is here, you have lateral organs coming, then this is your central zone. Auxins are getting transported to the primordia and then in primordia it is initiating program whereas, there is a feedback regulation here. But, if you remove organs the programming is totally disturbed as you can see from the PIN protein, the distribution of auxin is not happening in the normal way this is affecting meristematic activity as well as organogenesis.

Another important thing, so apart from that the organ specification or organ identity or differentiation one more thing which is getting ensured at this early stages organ polarity.

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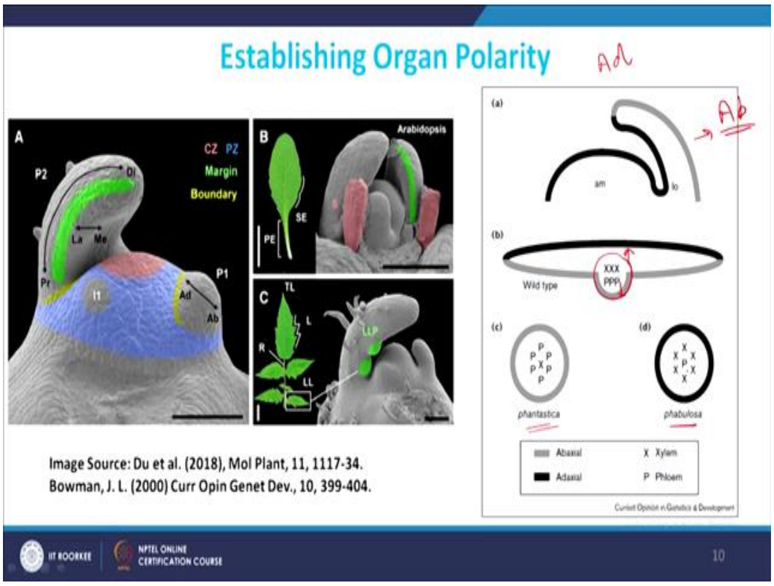

So, if you look any lateral organs whether it is flower or in different organs of the flowers sepal, petals, stamen or carpels or if you look the leaf they display polarity. Their basal side of the leaf which is very different than the apical side.

So, this kind of polarity is called proximal and distal polarity. On the other hand the part of the leaf which is towards the meristem is adaxial and the portion which is away from the meristem is called abaxial. And this polarity is also need to be established at the time of organogenesis. So, what happens in the growing meristem?

This is meristem and this is the lateral organ and in lateral organ the face of the lateral organ which is towards the meristem, this is called adaxial. And the part of them on the lateral organ which is away from the meristem is called abaxial, and if you look the features of adaxial and abaxial surface, it is very different. And this has to be established early. And in the leaf, if you look the leaf growing primordia. So, this is your adaxial side this is your abaxial side and if you look the positioning of the vascular tissue.

So, xylem is positioned towards the adaxial sides phloem is positioned towards the abaxial side and there are lot of genes which is responsible for maintaining this adaxial versus abaxial polarity. For example, if you have this mutants you can see that there is a radial pattern and you can see that xylem is in the center and phloem is the peripheral. If you look *phabulosa* mutant here it is opposite pattern you have a phloem in the center xylem outside.

So, in both the cases what is happening that polarity is disturbed. So, this polarity establishment occurs very early when meristem is getting maintained in the center and primordia and the lateral organ primordia is initiated.

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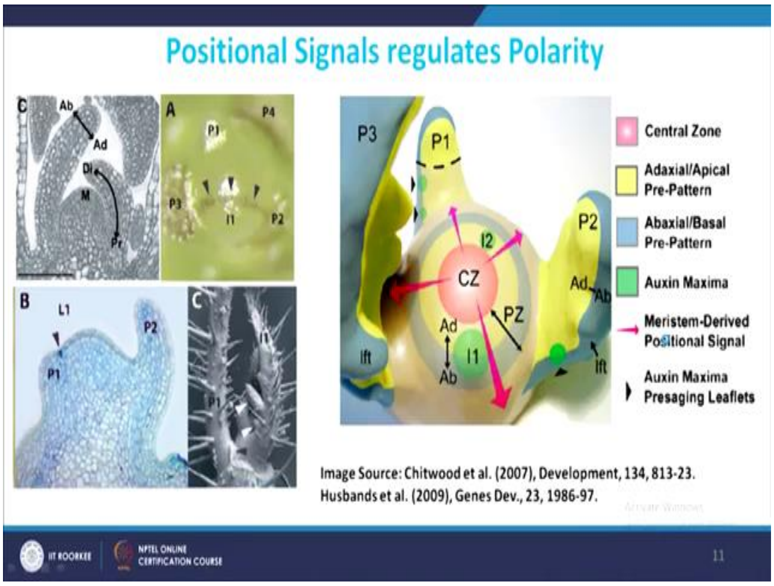

And the positional single plays very important role and this is clear from this experiment. This is a growing meristem and then you have a lateral primordia. This is the proximal region, this is distal region, this is your adaxial region, this is your abaxial region and what you do? If you just make a very small incision basically in just the L1 layer between primordia and the meristem. In L1 layer, what you see that the leaf primordia which is coming here it has totally lost the polarity.

It looks like more kind of radial patterning whereas, if you look this primordia which is coming from this side, it is still maintaining the polarity. You can clearly see the adaxial side is very different than the abaxial side whereas, here everything is looking like radial pattern. And this suggest basically that there is a communication between meristem and primordia that is also important for establishing organ polarity here.

So, if you look this schematic diagram, this is the top view you have a central zone and some kind of signals are coming from here and these signals are helping and defining adaxial versus abaxial polarity in the lateral organs.

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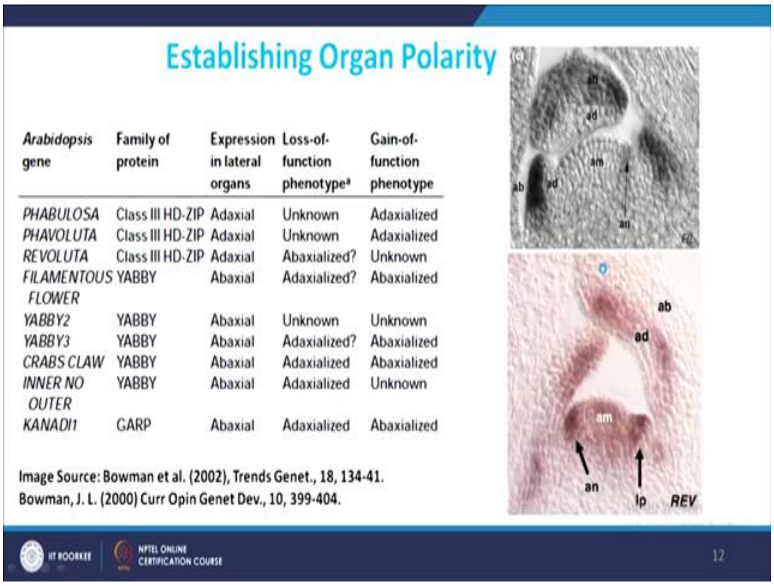

There are some of the genes which has been identified to regulate this organ polarity we will discuss in the next class. So, some genes are regulating adaxial surface some genes are regulating abaxial polarity.

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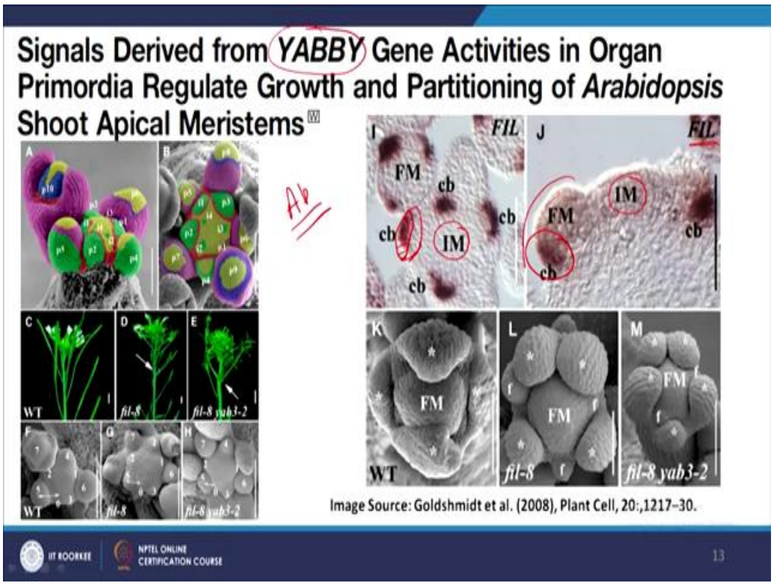

Another important thing here is that the signals derived from *YABBY* gene activity in organ primordia regulates growth and partitioning of *Arabidopsis* shoot apical meristem. So, it is not only that the meristem is regulating the organ, but the organ specific genes are also regulating the meristematic activity. So, *YABBY* class of genes are the genes which provides abaxial polarity in the organ. You will see in next class in detail. If you look the expression pattern of *FIL* which is a kind of *YABBY* gene, what you see that this is your inflorescence meristem expression in the inflorescence meristem per say is very very low or almost not detected.

But if you look the lateral organ primordia, the expression is very high, but expression is more towards the abaxial surface then the adaxial surface. If you look the top view of it you can clearly see that this is the primordia, but in the primordia expression is only restricted away from the meristem which is your abaxial surface. So, this suggest that these genes might be regulating abaxial fate.

And, what happens in this mutant background if you look this is a *filamentous* and the double mutant *filamentous* and *yabby3* mutants, what you see that the defect in the organ polarity genes is basically disturbing the phyllotaxis. The arrangement of the organs which is a feature of meristematic activity.

So, this is visible from here if you look, the distance between two adjacent primordia is here in the wild type, but in the mutants this distance is basically changed. So, this suggest that the polarity genes which are expressed in the organ they are also regulating the meristematic function in the shoot apical meristem.

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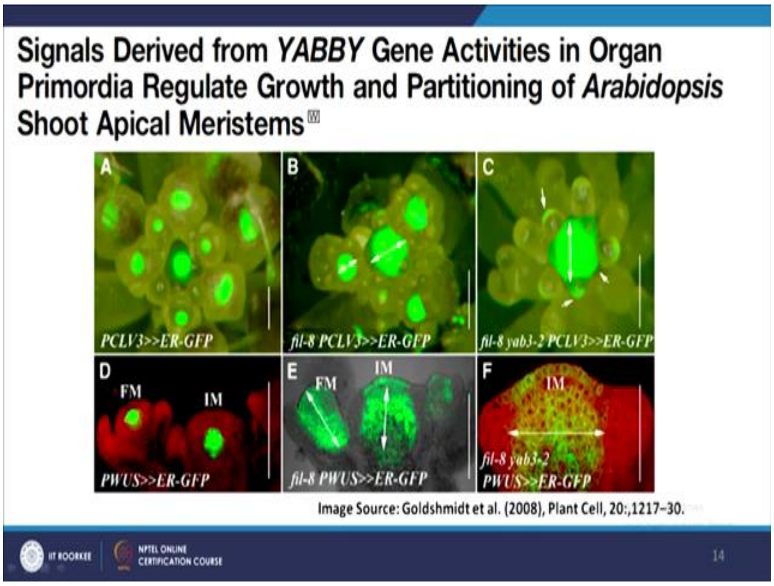

Two very important and well established meristematic markers are *WUSCHEL* and *CLAVATA*.

In wild type this is the *CLAVATA* expression domain and this is in the very tip restricted here in the center, but if you look the single mutant the domain of *CLAVATA3* expression is expanded, and double mutant it can be even further expanded. Similarly if you look *WUSCHEL* expression domain here in the wild type it is restricted in the few cells here. But if you look the single mutants or double mutants the *WUSCHEL* expression domain is significantly expanded both laterally as well as in the apical and basal domains.

So, this suggest that the meristematic activity or the regulators of meristematic activities their expression pattern and their localization is disturbed when you have defect in the organ polarity.

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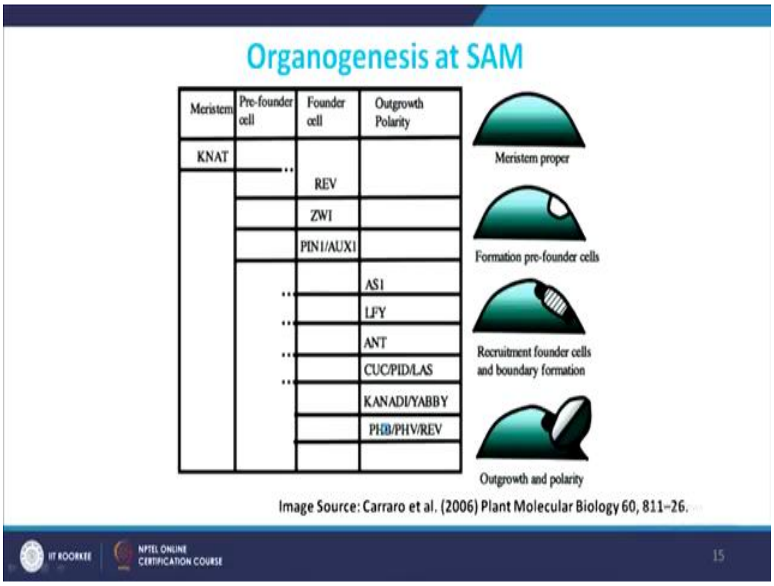

So, there is a some of the genes which is regulating meristematic activity, and then in the peripheral zone the founder cells has been specified where auxin and some of the regulators are playing very important role and then outgrowth and polarity is regulated.

So, these are the three different steps during the process of organogenesis and there are some genes for example, *ASL*, *LEAFY*, *ANTIGUMETA*, *CUC*, *KANADI* regulating the process together.

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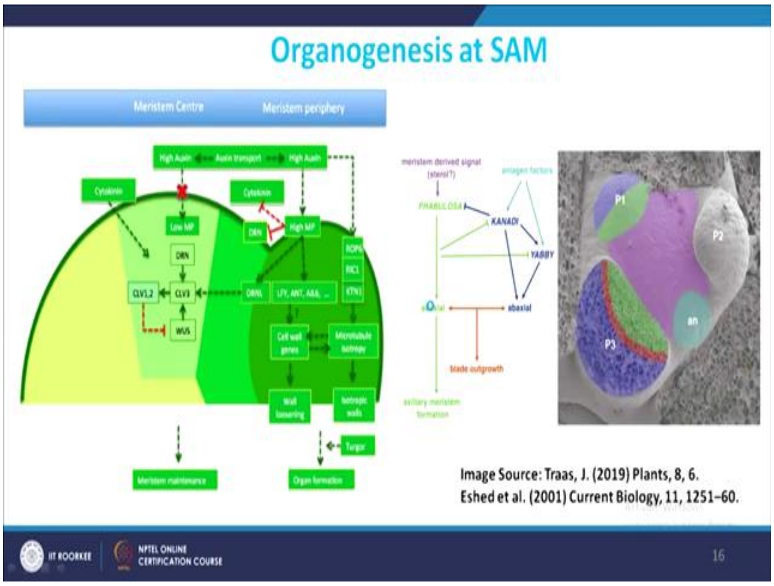

In the meristematic zone, where you can see *CLAVATA* and *WUSCHEL* mediated signaling and cytokinin along with many other genes as well they are regulating the meristematic activity.

But in the peripheral zone, in the primordia you have high amount of auxin; auxin is regulating some of the transcription factor and this transcription factors are basically activating, organ specific genes *LEAFY*, *ANTIGUMETA*, like genes and these genes are ensuring a proper differentiation. At the same time there are some signals, which is derived from the meristem. And they activate some of the gene like *PHABULOSA* in the adaxial side of the lateral organs.

And some other genes are getting activated in the abaxial side of the lateral organs and there is a crosstalk and regulation between adaxial and abaxial genes. And this regulation is basically ensuring a proper polarity in the growing lateral organs. So, I will stop here in next class we will discuss leaf development.

Thank you.