Plant Developmental Biology
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Lecture - 17
Shoot Development: Leaf Development

Welcome back to Plant Developmental Biology. We are continuing Shoot Development and particularly organogenesis during shoot development.

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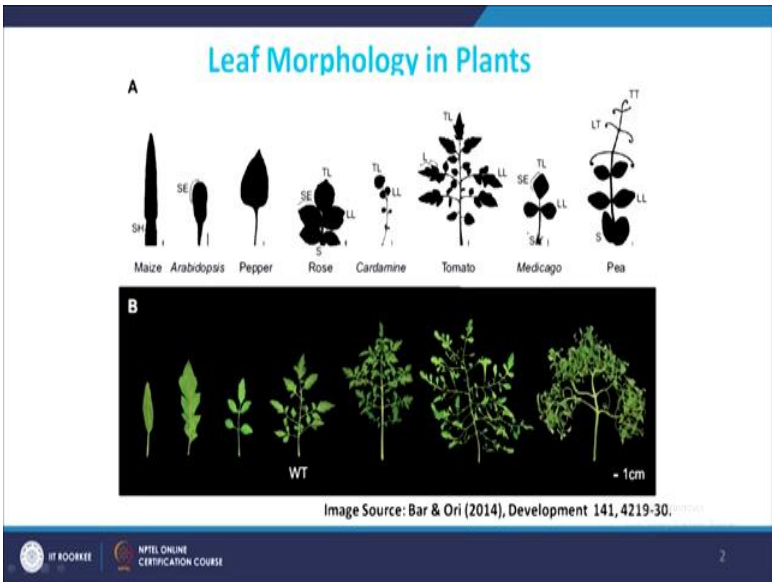

So, in previous class we have discussed the coordination between shoot apical meristem maintenance as well as the organogenesis in the peripheral region. In this class we are going to take example of leaf development. You know that during vegetative growth the major organs which a plants make is leaves and there is a huge variations in the morphology of the leaves across the plant species. You can have different plants having a different shape, size and arrangements of the leaves. For example, if you look maize, *Arabidopsis*, rose, there is a huge variety in the morphology of leaves.

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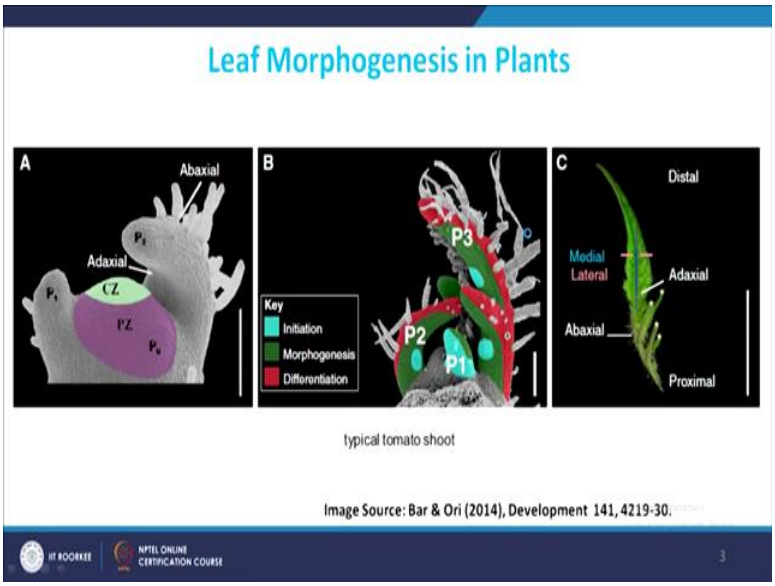

But if you look the pattern of their development, there is a quite similarity with some species specific differences. But what happens generally during the leaf development, as you have seen in the previous class that the meristem is being maintained here in the center. And then in the peripheral region the primordia is getting positioned.

So in case of leaf development the primordia is going to be leaf primordia and this is positioned by generating auxin maxima in the peripheral region. And once the auxin maxima is generated here, the next auxin maxima will be generated at a particular distance from the existing leaf primordia. And then like that you see early primordia patterning and differentiation.

Once the primordia is positioned it will start the leaf specific program and then during the differentiation it will make all the tissues, which are specific for the leaf, it will ensure the polarity in the leaf which is adaxial and abaxial and the proximal and distal polarity. This is a typical picture of tomato shoot. You can see that this is meristem this is the leaf primordia 1, P2, P3 and then these are the morphogenesis. These regions are basically differentiating and eventually they are giving mature leaf where you have different part of the leaf.

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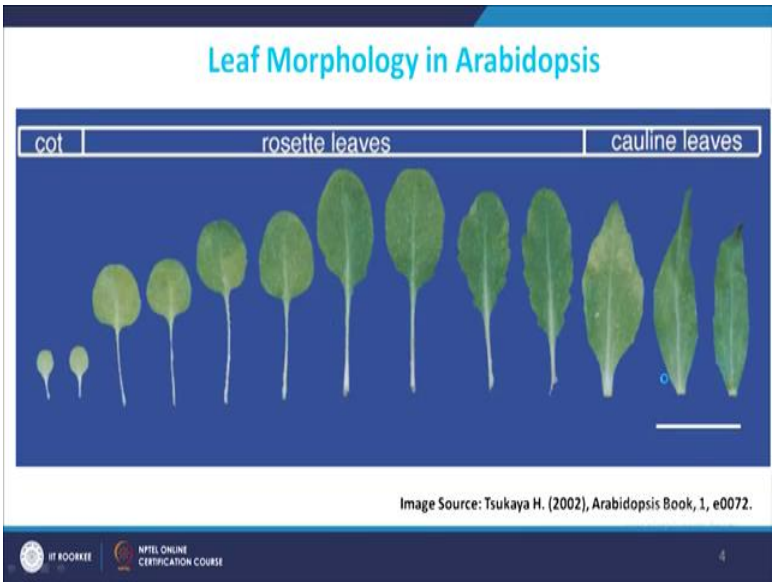

This is a leaf morphology in model dicot plant *Arabidopsis*. During early stage of the development they have cotyledon type of leaf, which are very small. During vegetative growth they makes rosette leaves. Rosette leaves show a variation in the shape size and morphology.

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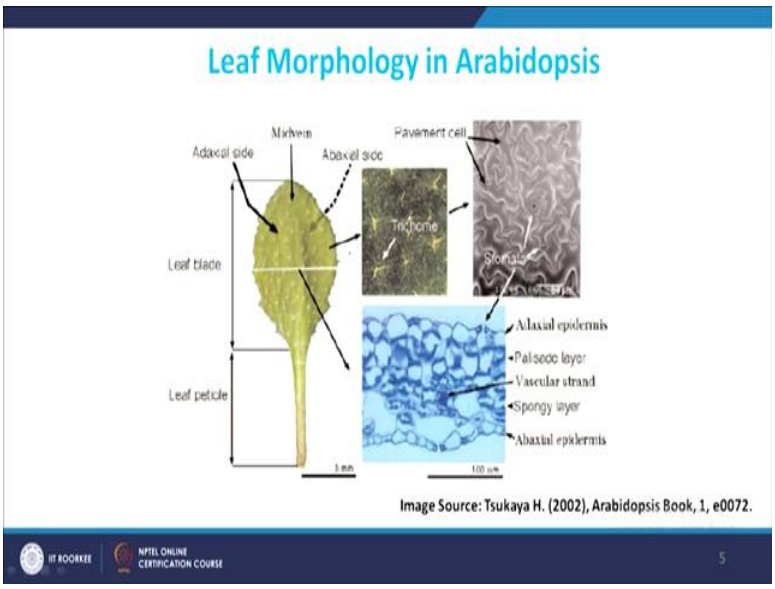

And then you have a cauline leaves. These kind of leaves are usually produced in the inflorescence stem after transition. In leaf you have leaf petiole and this region is called leaf blade. It has midrib and adaxial, as well as abaxial sides. On one sides you can

clearly see that trichomes are developed and then you have stomata formation and this is a cross section of the leaf. At adaxial side you have palisade cell then you have in between the vascular tissues, spongy cells and then you have the abaxial sides and during the development what happens that, the primordia or the program which is specific for the leaf development is getting positioned at the site of the leaf formation. And then during the differentiation these tissues are patterned, and taking a special identity and then eventually the growth a leaf is basically restricted.

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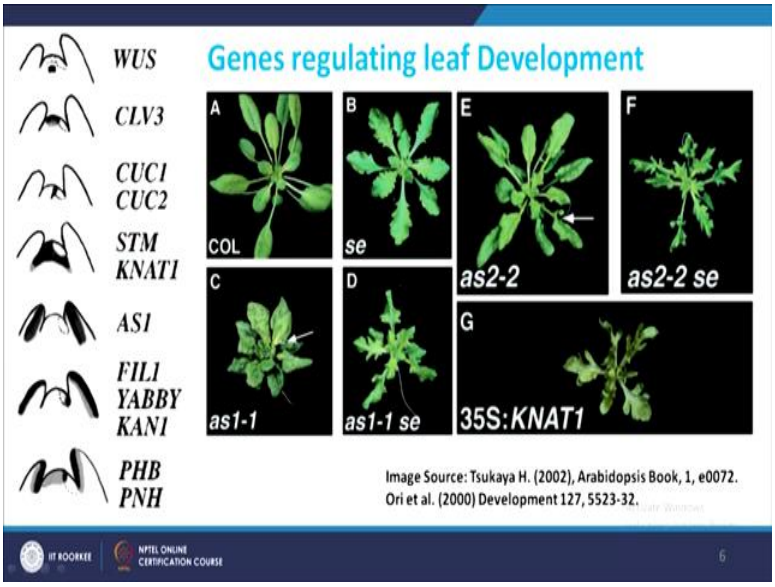

So, these are the some genes, which is known during leaf development. So, *WUSCHEL* and *CLAVATA3* they are responsible for meristem maintenance then you have *CUC2*. *CUC2* is as I said that *CUC1* and *CUC2*, they are the boundary genes they express very specifically, at the boundary of shoot apical meristem and leaf primordia. So, they are basically maintaining the boundary then, you have *STM* and *KNOX* gene which is regulating the meristematic activity.

You have some other genes like *ASYMMETRIC1* which is regulating the leaf patterning. And then you have *YABBY*, *FILAMENTOUS* and *KANADY* genes which is regulating abaxial polarity *PHABULOSA* and other *FANTASTICA* is a gene which is regulating adaxial polarity.

So, these genes are getting expressed and then they are regulating the function. And if you have mutation in these gene you are going to see the defect in the leaf morphology.

For example, if you look this mutant which is *serrate* mutant, so wild type this is the morphology of the leaf you have petiole, then you have the blade. But if you look here there is a serration, there is a kind of change in the morphology of the leaf.

Similarly, if you look *asymmetric2* mutants you have a similar kind of phenotype, but phenotypic severity is not very high. But if you make a double mutant between *as2* and *serrate* or *as1* and *serrate* you can see the phenotype is severe. Similarly, if you take some of this *KNOX* gene and if you ectopically over expressed you can see that leaf morphology is totally disturbed.

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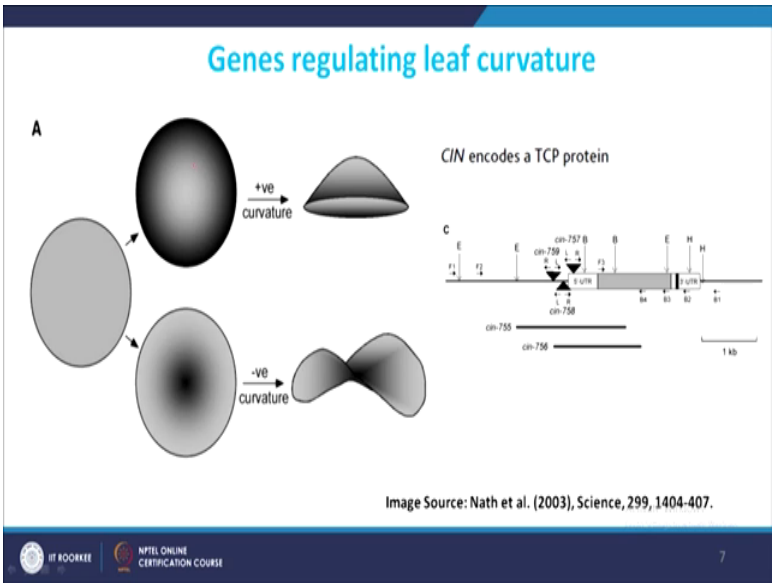

Another things what defines the leaf shape and size is leaf curvature.. So, how curvature is maintained. If you take a cell and if the growth occurs uniformly then you have a cell growth in a more kind of spherical manner. But if growth in the central region is more, than the peripheral region then you get positive curvature and you can see a structure like this, a curve like this.

On the other hand if growth is more in the peripheral region, then the central region then you will have a kind of negative curvature and then you can have this structure. A mutant of *Antirrhinum CINCINNATA* genes has been identified. *CINCINNATA* gene is a class of transcription factor of TCP family and what it was found that if you look this mutant the morphology of leaf is extremely defective.

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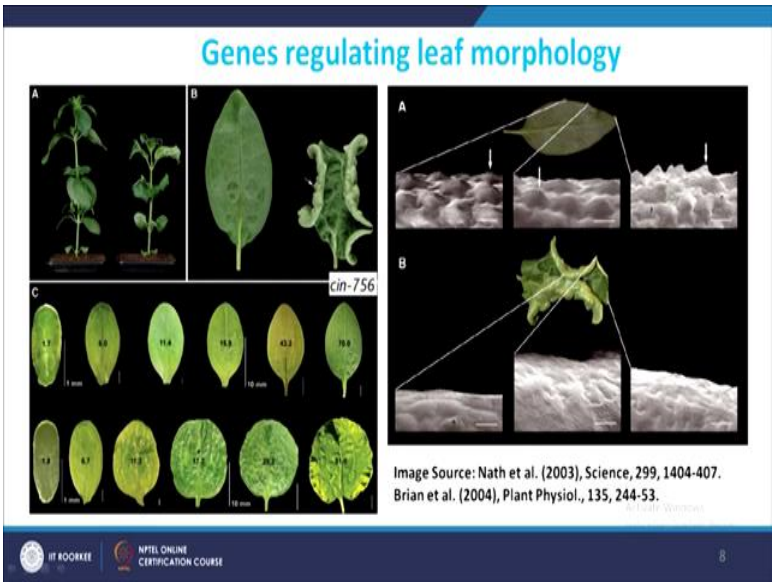

So, this is a typical *Antirrhinum* leaf you have a petiole and there is a proper shape. But in *CINCINNATA* mutants what you see that the curvature is totally. If you look the defect in the leaf you can clearly see that at the early stage difference is not very prominent, but at the later stage this leaf when spread you see this kind of structure. If you look the margin of this leaf and if you see the cellular feature of this leaf you can clearly see that in wild type leaf and the *cincinnata* mutant's leaf, has very different feature of the margin cells.

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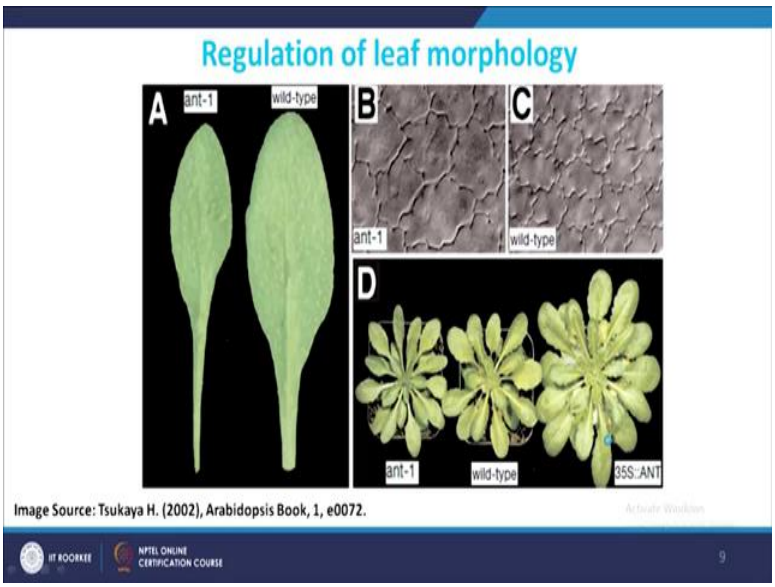

Another genes which are known to regulate leaf morphology is *AINTEGUMETA*. If you look here this is a typical wild type *Arabidopsis* leaf, but if you have loss of function of *ANT* you can see that shape is somewhat similar, but size of this leaf is totally disturbed. And if you look this is a wild type pattern if you have loss of function of *ANT*, you see this defect. If you have gain of function of *ANT* if you have over expressed you can see the opposite effect here. So, this suggests that *ANT* is also regulating leaf morphology.

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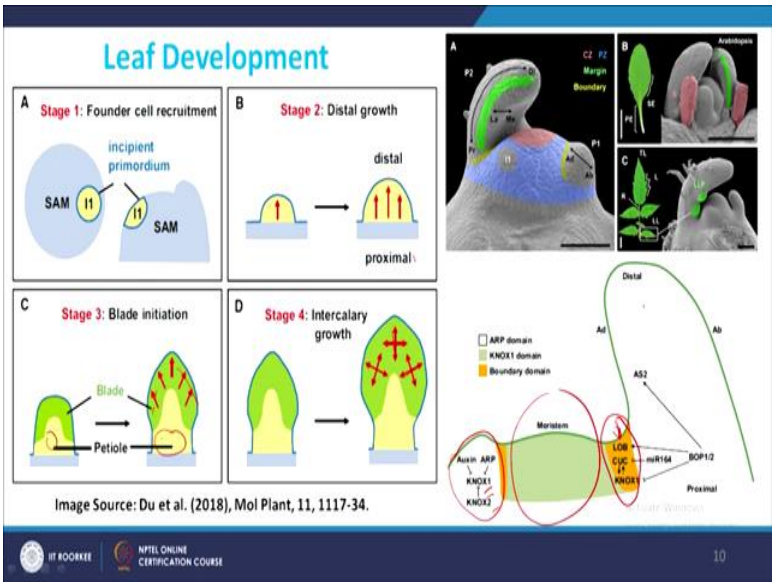

So, in general if you look the development of leaf, in the first step in the shoot apical meristem or at the peripheral region of the shoot apical meristem the first leaf primordia is positioned. And then this leaf primordia start distal growth which occurs in this direction. This generates a kind of two end, one is the proximal and the other is distal region. And then at third stage the blade initiates where the marginal region or this region is basically getting developed into the blades and the basal reasons are basically developed into the petiole.

Later on there is an intercalary growth it start in the blade region and that basically helps in the leaf blade to take a proper structure. And this is the same schematic diagram or picture where you can clearly see developing primordia, this is early primordia, this is slightly later primordia this is the leaf primordia which is at this stage of coming out.

And there are some genes which has been identified to regulate the process. In the meristem the meristematic genes are there which regulate the meristem function. But in

the primordia you have other genes which are getting activated. One is the auxin level and auxin inhibits the meristematic gene. So, basically in the primordia region meristematic genes or the genes which are basically negatively regulating the differentiation program need to be suppressed.

At the same time the genes which are positively regulating the leaf specific developmental program they need to be activated. And LOB kind of genes are the genes which are present at the boundary between meristem and the leaf primordia; and they are very important to defining the zone or the specially positioning the meristem or separating meristem with the lateral organ primordia. At the same time you have a set of genes, which are getting activated in the developing leaf primordia and these genes are important for regulating leaf specific differentiation program.

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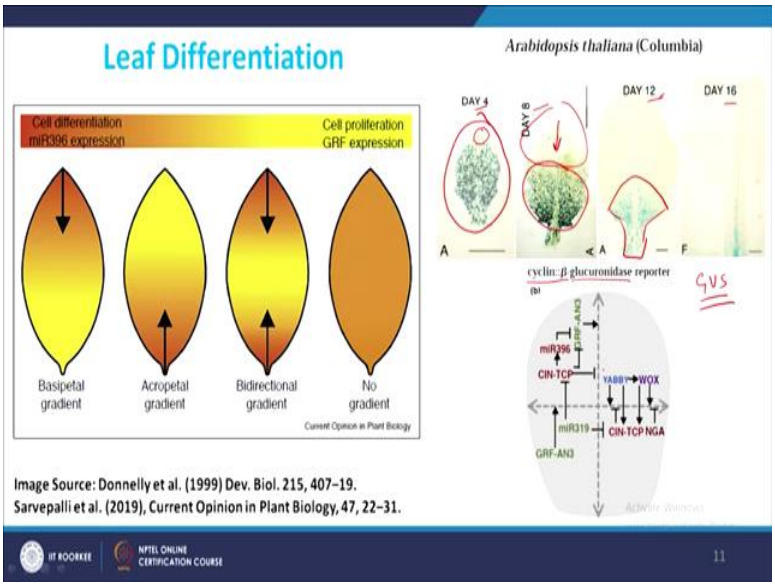

So, after this there is a process of differentiation, it can be either basipetal, acropetal or bidirectional which means that differentiation can start from here and proceed in this direction or it can start from the basal region and proceeds in the upper direction or it can starts from the both direction.

And then it generates a kind of gradient, but once both the ends are completed then you have no gradient, when there is no gradient essentially you can say that here the differentiation or growth of the leaf stops. So, if you look *Arabidopsis thaliana* so in *Arabidopsis thaliana* it occurs in the basipetal manner.

This is *Arabidopsis* leaf at different days this is day 4, day 8, day 12 and day 60 and this is a *CYCLIN* promoter driving *GUS*. So, *CYCLIN* is a gene which basically marks the dividing cell; it is a cell cycle gene. So, if you look at the very early leaf which is around 4 day old, you can see the division occurring almost entire region of the leaf.

So, this suggest that differentiation is only restricted at the very tip of the leaf. Whereas, most of the leaf they are having cell division or proliferation activity going on. But when this leaf becomes older, so if you look in the 8 day old leaf what happens that the differentiation signal is migrating towards the down side. So, if you have now around 50 percent of the tip region they do not have cyclin activity.

So, they are not showing the division activity, which means that the division activity is mostly restricted to the basal half region of the leaf whereas, the apical half region or the distal half region of the leaf basically they lack the division activity, which means that they are undergoing the process of differentiation. Then if you see at more later stage like 12 days the division activity is restricted only in the very basal region of the leaf; whereas, most of the distal region of the leafs are showing pattern of differentiation.

Slightly more later if you look 16 days old plant the entire leaf they lack the cell division or cell growth activity; which suggest that now this leaf is fully mature it is fully differentiated now it will stop the property of differentiation. And then some of the genes has been identified which regulates this property.

If you divide this leaf so there is apical and basal or distal and proximal growth of the leaf then you have this marginal growth. Some of the micro RNA, some of the GRF or the growth regulating factors and *CINCINNATA*, *TCP* genes, the activity of these genes basically ensures proper shape, size, and tissue patterning during leaf development.

On the other hand if you look the activity of *YABBY,* homeodomain transcription factor *KANADI* genes, they basically ensures the adaxial versus abaxial property or polarity of the leaf.

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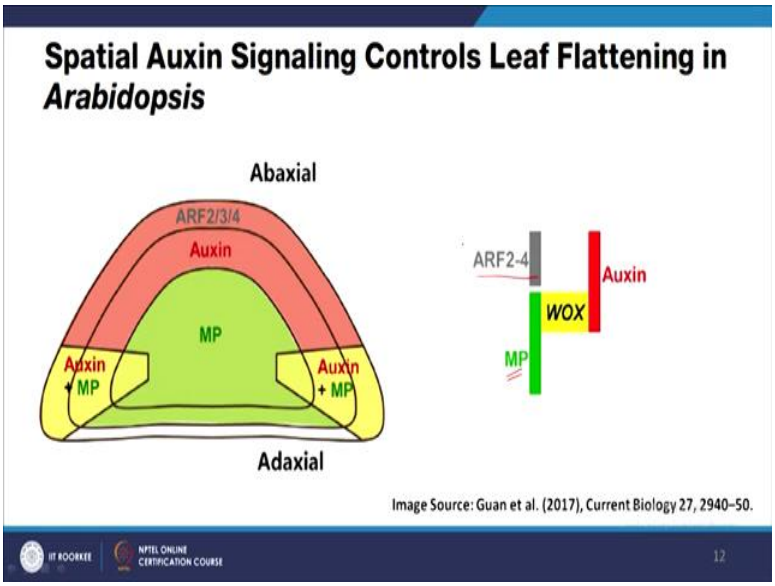

Auxin is known to regulate leaf flattening in *Arabidopsis.* Auxin regulates activity of *AUXIN RESPONSE FACTORS*. So, these are *AUXIN RESPONSE FACTOR2* to *4*. Then you have *MP* which is again *AUXIN RESPONSE FACTOR 5*, then some of the *WOX* genes and their activity together basically ensures not only the leaf differentiation or leaf initiation, but also it regulates leaf flattening in these direction.

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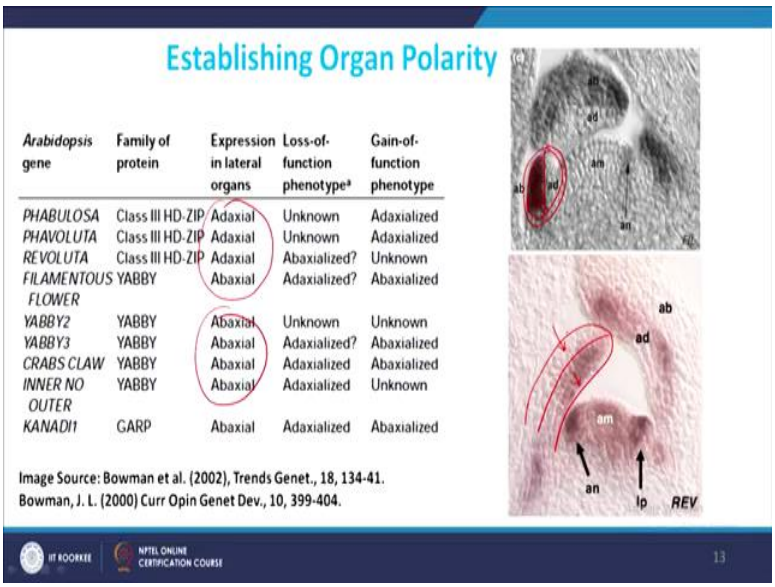

Now, we will look the leaf polarity. So, as I said that leaf polarity is also very important and because some of the features which are present in the adaxial surface is not present

on the abaxial surface and so polarity is important. And polarity is regulated by a clear two category of the genes. So, one category of the genes which are specifically expressed at the adaxial side of the lateral organs, another category is expressed specifically on the abaxial side of the development.

For example, if you look this growing pattern and this is messenger RNA distribution of *FIL* gene which is abaxial gene. This is developing leaf you can clearly see that expression is mostly restricted the abaxial half of the leaf whereas, the adaxial half portion of the leaf do not have this expression. And this expression pattern is established very early even at the primordia stage, so this is your meristem and this is your first primordia here coming.

This region is the primordia, but expression is only restricted to the abaxial half of the primordia, but half adaxial primordia totally does not express this abaxial genes. Whereas, on the other hand or in contrast to this if you look the adaxial genes, which is like *REVOLUTA* or *PHABULOSA* you can see it is showing an opposite pattern.

So, if this is a growing leaf primordia, you can see that the half primordia which is towards the adaxial surface express these genes very high. But the abaxial side of the primordia it does not have the expression. The asymmetric expression pattern or transcript distribution of these genes essentially, provides a proper polarity in the developing leaf.

Apart from the homeodomain containing transcription factor in abaxial you have *YABBY* domain transcription factor, *KANADI* and some of the other genes. In this mutant such as *revoluta* you can see that loss of function phenotype is where adaxial surface is totally abaxialized.

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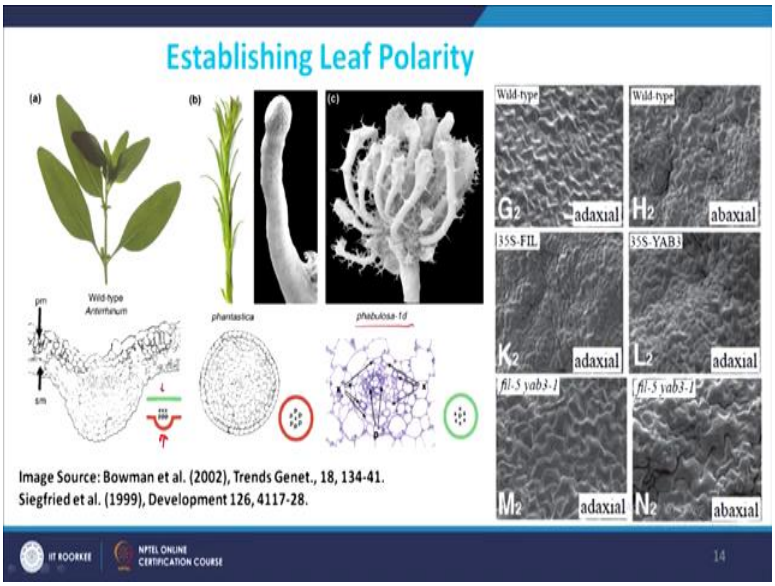

This is wild type *Antirrhinum* leaves, but if you have a *fantastic* a mutant *FANTASTIC* is a regulator of adaxial surface. If you do not have this gene expressed you can see that the polarity in the leaves are totally lost; adaxial surface and abaxial surface both looks quite similar. And if you make a cross section for the wild type, this is your adaxial side, this is abaxial sides and you can see that xylems are usually position towards the adaxial sides phloems are position towards the abaxial sides.

But if you take the mutant and make a cross section it shows radial pattern. And here phloem is in both the sides and xylem is in the center. This suggest that when you do not have adaxial specific genes the all tissues are getting abaxialized, so you have abaxial type of pattern in the leaf. On the other hand if you have *PHABULOSA* dominant, *PHABULOSA* is again regulator of adaxial surface. But here it is a gain of function mutant so, if you have gain of function mutants then what happens that the all the regions are getting adaxialized.

So, even abaxial regions get converted into adaxial region and here the vascular pattern is very different it is opposite to the loss of function here. You can see here xylem is surrounding the phloem which means that xylem is basically adaxial side. So, in this mutant background you can see that leaf is mostly adaxialized whereas, in loss of function the leaf is mostly abaxialized. If you look the cellular feature of the adaxial

versus abaxial surface in scanning electron micrograph adaxial surface abaxial surface looks different.

On adaxial surface you can sees that there is a bulges of the cells whereas, if you look the abaxial surface it is relativity smooth. So, bulges are not very strong, but if you ectopically overexpress *FILAMENTOUS* or *YABBY* gene which are regulator of abaxial surface. They promote abaxial surface. Adaxial surface normally in wild type looks like this; you have lot of bulges, but when you over express or you put *FILAMENTOUS* or *YABBY3*, they becomes more similar to the abaxial surface. They are more smooth.

This suggest that when you have a gain of function activity of *YABBY3* or *FILAMENTOUS*, they are converting adaxial identity to abaxial identity, but if you have the loss of function like a double mutant of *FIL* and *YABBY3*.

So, here both the abaxial promoting genes are lacking. In that case even abaxial surface look very similar to the adaxial surface.

So, you have clear two category of genes the adaxial promoting genes and abaxial promoting genes if you do not have adaxial promoting genes adaxial sides get converted into abaxial sides, if you do not have abaxial promoting gene abaxial surface get converted into adaxial surface.

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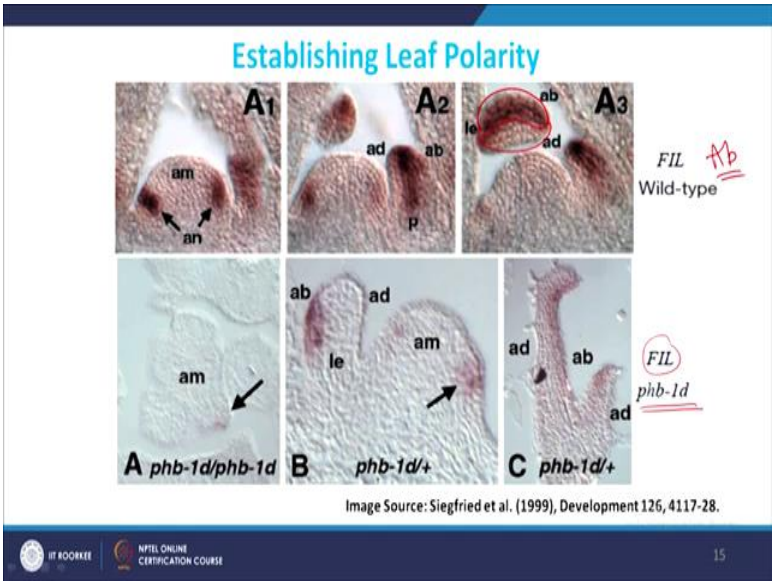

And they are actually regulating their activity as well. So, if you look here, this is the expression of *FIL* which is abaxial gene it is mostly expressed in the abaxial side of the leaf and it is absent in the adaxial side. But if you have gain of function mutant of *PHABULOSA*, means every side is adaxial side and this is the expression pattern of *FILAMENTOUS* gene because *FILAMENTOUS* is abaxial.

Now, in this mutants you have everything adaxial, you can clearly see that if you have a homozygous gain of function mutants of *PHABULOSA-1d* you can see the expression is almost disappeared. So, there is no expression of abaxial specific genes. On the other hand if you look here in some of the cases the expression is there, but it is very restricted it is not the expanded. So, its domain of expression is highly restricted and if you look some of the organs which are basically adaxialize you can clearly see that their expression is very low and extremely disturbed expression pattern.

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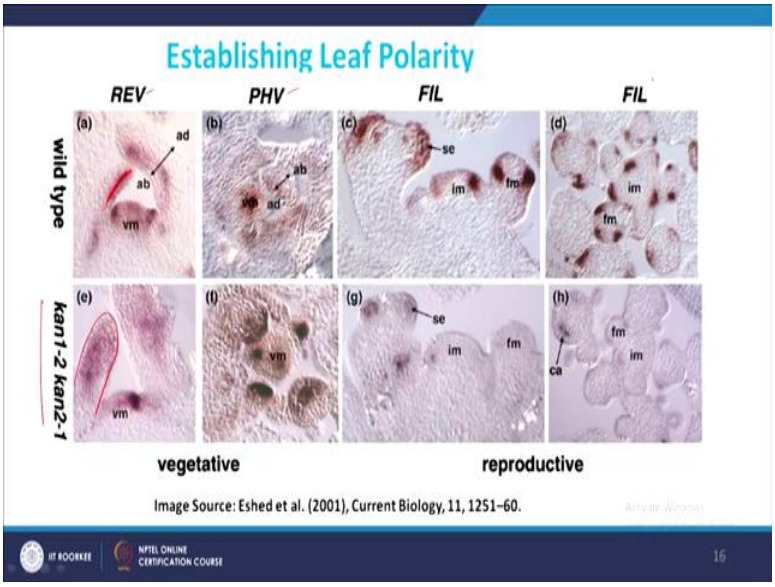

On the other hand, if you look the expression pattern of adaxial promoting gene in the abaxial mutant. *KANADI* genes are basically responsible for promoting abaxial side. Now if you have double mutant here and if you look now *REVOLUTA*, *PHAVOLUTA* these two of genes are adaxial side genes you can clearly see in mutants background their pattern is totally disturbed.

In the wild type they are clearly localized towards the adaxial surface, but if you look the mutant here you can see they are quite uniform or you can see even expression in the

abaxial surface. And similarly if you have *kanadi* mutants the abaxial genes they have lost their expression or they are very low level expression.

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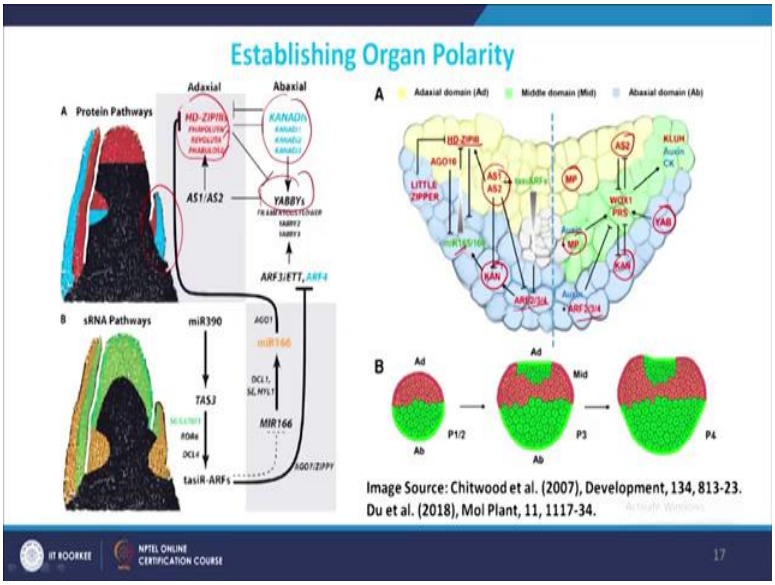

If we summarize, in the growing leaf you have the meristematic zone and the primordia. In the primordia, the adaxial versus abaxial polarity is already established. And in adaxial side these homeodomain containing transcription factor, which is *PHABOLUTA*, *REVOLUTE*, *PHABOLUSA* they are active. On the other hand *KANADI*, *YABBY* these genes are active on the abaxial surface and they mutually inhibit each others in their domain.

In adaxial domain these genes inhibit expression of *YABBY*, in abaxial domain these genes inhibit expression of homeodomain gene. And these kind of mutually exclusive expression pattern of adaxial promoting genes and abaxial promoting genes establish the adaxial versus abaxial polarity. On the other hand there are some of the micro RNAs, trans-siRNAs they are also playing a very important role in establishing and regulating, polarity in these organs. If you look the cross section of the leaf, you can clear see that you can have a two domain; the adaxial domain and the abaxial domain.

So, these are the adaxial domain where adaxial genes like these are active and apart from the adaxial genes you can see that genes which are responsible for the promoting leaf identity they are also getting expressed. On the abaxial side you can have some of the abaxial promoting genes, which are getting active; apart from that you have the activity

of auxin, micro RNA, *AUXIN RESPONSE FACTORS*, *MP* and some of the other key regulators of leaf differentiation. So, all together activity of hormones activity of transcription factors the coordination between meristematic activity and the leaf primordia set complex genetic program which is regulating proper patterning, differentiation and organ development in plants. So, I will stop here in next class we will look floral transition and flower development.

Thank you very much.