Plant Developmental Biology
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Lecture - 18
Shoot Development: Flowering

Welcome back to Plant Developmental Biology course. In today's class we are going to discuss about Flowering. So, flowering is basically a transition from vegetative phase to the reproductive phase in the life cycle of a plant.

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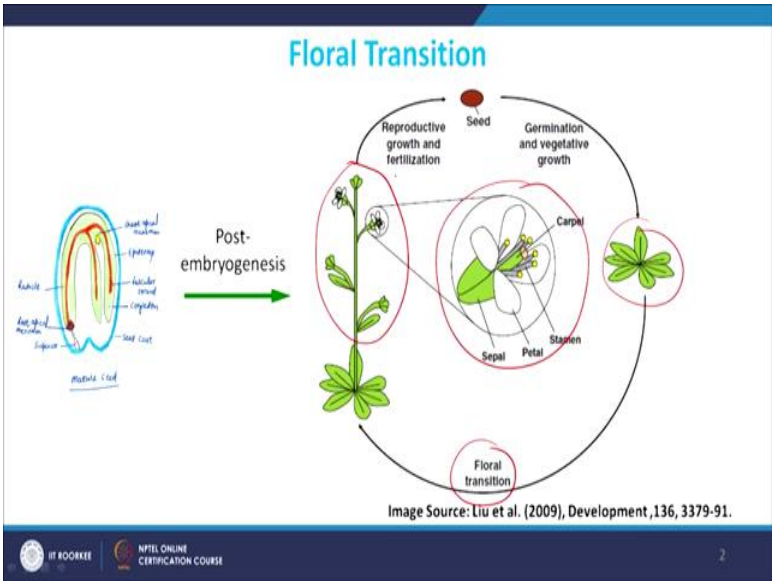

So, when your plant start post-embryonic development after seed germination at a certain time period or up to certain period of time in the life cycle they only continue the vegetative growth.

And that is the period when they make branches they make leaves and then at a particular time point in their life cycle a major decision is being taken place; where this vegetative plants enters in the process of floral transition and it start the reproductive phase; and this is very important in the life cycle of the plant because, reproductive phase of the life cycle ensures the sexual reproduction. And sexual reproduction is one of the highly evolved mode of propagation in case of higher plants.

The sexual reproduction is restricted in an organ or in a part of the plant which is called flowers, which are developed on a structure which is called inflorescence.

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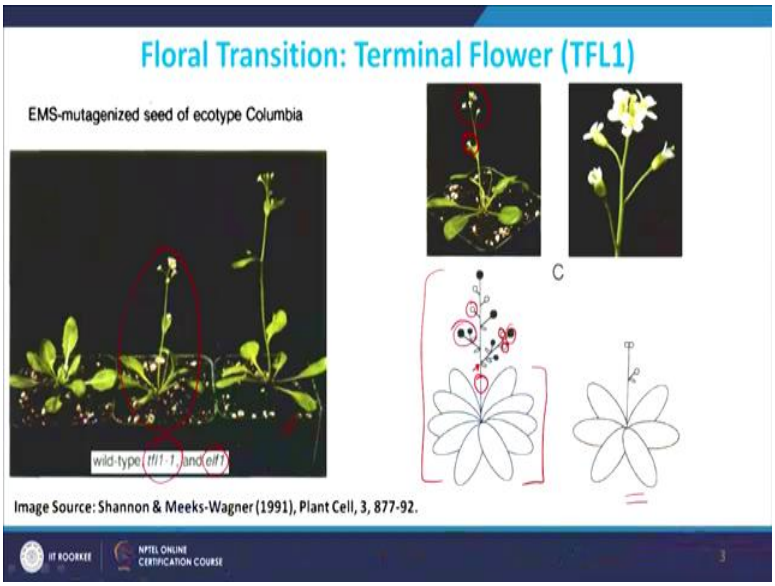

In today's class we are going to discuss how this transition occurs, what are the genetic and environmental factors which regulates the timing of the transition and ones this transition has ensured, how a special organ which is flowers with a special structure organs like sepal, petals, stamens and carpels are developed.

It is very important to understand that during the vegetative process reproductive phases are repressed; and it is important because, plant has to gain a certain amount of strength before it enters in the process of reproduction. There was a gene identified which is called *TFL1*, *TFL* is basically *TERMINAL FLOWER1* and a mutant was identified in this gene where the plant flowered early.

If wild *Arabidopsis thaliana* takes about 40 days to flower this mutant plant will flower much before. In the terminal flower mutants, it flowered early, and these branches are getting terminated with a single terminal flower.

In the life cycle of model dicot plant *Arabidopsis* during the vegetative phase these leaves are formed which is called rosette leaves. Then after transition the shoot apex get converted into inflorescence meristem and this inflorescence meristem has a mixture of property.

The branches which are coming from the inflorescence meristem, they have indeterminate nature. Whereas, on the branches if you look this floral organ or flowers they are determinate in nature. The branches can grow and they can make many flowers, but once a flower is made the meristematic activity is terminated. But in the *tfl* mutant this shoot like structure get terminated with a single determinate flower.

This was the phenotype which was observed in *tfl1* mutant. This is the top view and you can see that here the growth basically stops in a way and not many flowers are made in this case. So, this is important which suggest that *TFL1* could be one important gene which basically repress the flowering which is a negative regulator of flowering.

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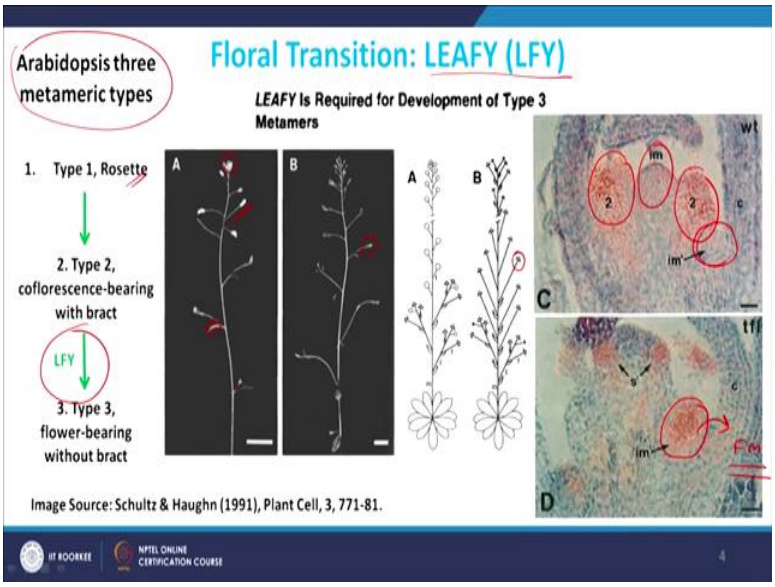

On the other hand if you look another gene or another mutant which is called *leafy*, *LEAFY* is activator of flowering. In the *Arabidopsis thaliana* plant there are three types of structure; type one rosette and then there is a transition from here to here this is the transition which we call about the floral transition. And here the first few structures which you look like here, here they are called coflorescence; they bears a bract like structure. And then if you come to the higher side there is a flowers which is without bract like structure.

So, this is the typical architecture of *Arabidopsis* inflorescence. In wild type you have this co-florescence and then you have this final flowers, but in case of *leafy* mutant the co-florescence are fine, but the later flowers which are getting converted like shoot like structure.

So, there is a loss of floral identity, which suggest that *LEAFY* is important. If you consider these two processes the entire and complete process of transition from vegetative shoot apical meristem till the flower which has two stages and this final stage is regulated by *LEAFY*. The expression of *LEAFY* in the inflorescence meristem which is on the apex is very low or not detectable. But if you look the flowers or floral primordia

which is originated at the peripheral region of the inflorescence meristem, it has a very high amount of *LEAFY* expression.

This suggest that the *LEAFY* gene is very important in giving identity to the floral meristem when you do not have *LEAFY*, this meristem is not acquiring floral meristem identity instead of that it looks more kind of inflorescence meristem identity. And if you look this side the expression of *LEAFY* is very low.

But in *tfl* mutant this side, the region from where your branches are coming the expression of *LEAFY* is very high. And that is important, because when you have high amount of *LEAFY* expression it will convert this meristem to floral meristem identity.

This also proves that *TFL* and *LEAFY* they looks opposite. So, *TFL* is may be more important for giving shoot identity or shoot like structure inflorescence identity. Whereas, *LEAFY* is important for giving identity to the floral meristem and the critical balance between them basically ensures the time of transition and the identity of a meristem.

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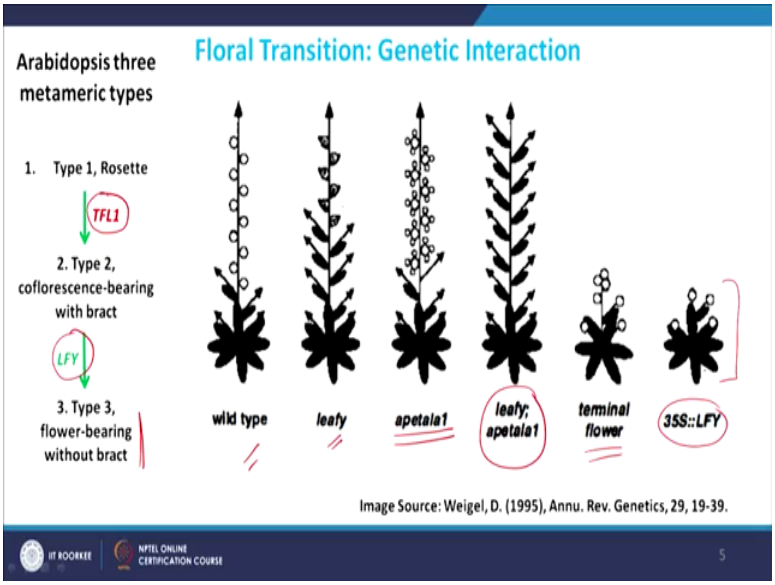

So, *TFL* is regulating even the first step of transition. And then *LEAFY* is ensuring a proper floral meristem identity, in the peripheral region and these are the typical schematic diagram of other mutants. So, this is wild type and if you look the *leafy* mutant you can see that the there is a defect in the floral meristem identity.

Another gene which is *APETALA*1 which is *AP1*. This is another important gene which is responsible for giving floral meristem identity and it together with or in parallel with

LEAFY works to ensure the floral meristem identity. And important thing that if you have double mutant of *LEAFY* and *AP1* you can see that phenotype of both *LEAFY* as well as *AP1* mutant phenotype is enhanced which suggest that they are working in two parallel pathways. And this is your terminal flower when you have *terminal flower* mutant you have a terminal flower. And if you over express *LEAFY* it looks like that it is mutant of *terminal flower*.

So, all these interaction basically suggest that *LEAFY* and *AP1* they are important factor in giving floral meristem identity to the peripheral meristem developing at the inflorescence and *TFL* is a negative regulator of floral meristem identity.

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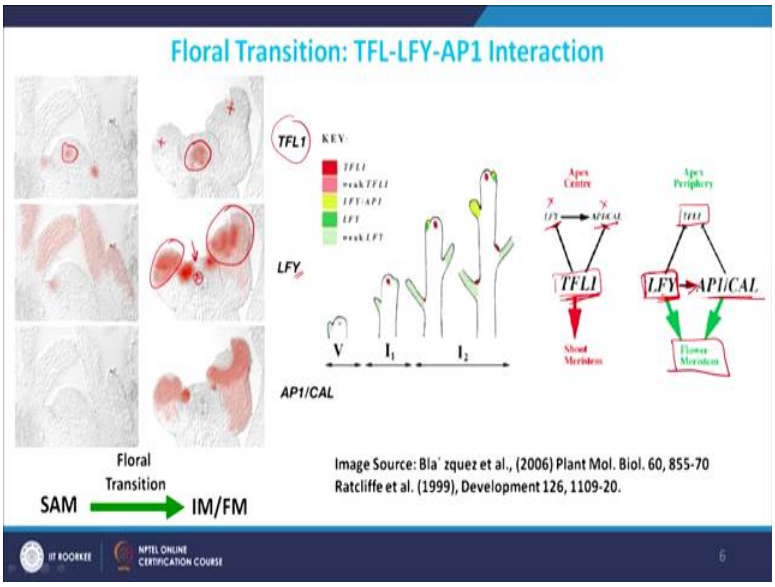

If you summarize the expression pattern of *TFL1* during vegetative phase is here, here, but in the reproductive phase after floral transition it continue expressing in the inflorescence meristem. But none of the floral meristem are developing floral primordia or show any expression of *TFL1*.

Whereas, the inflorescences meristem or the center of meristem does not have *LEAFY* expression, but this peripheral region where your floral primordia is going to be initiate they have very high amount of *LEAFY* expression and this expression continues in the developing floral primordia.

AP1 has a quite overlapping expression pattern like *LEAFY*. In the very early floral primordia you have *AP1* expression, then at the developing floral primordia you have a high level of expression at the later stage the expression is restricted to the first whorl organ which in case of flowers is sepals.

So at the center you have high amount of *TFL1* expression and you have very less amount of *LEAFY* expression. And when you have high amount of *TFL*, *TFL* goes and it repress both *AP1* as well as *LEAFY* in the center region. And since you do not have *LEAFY* you do not have *AP1* which means that you cannot assure any floral meristem identity in the center so that is it this helps in maintaining inflorescence meristem identity at the apex.

But if you look the peripheral region just here in the peripheral region your *LEAFY* is getting activated and when *LEAFY* is getting activated, *LEAFY* also activates the expression of *AP1* and then both *LEAFY* and *AP1* together specify or provide floral meristem identity to the peripheral meristem.

On the other hand they also repress *TFL1* in the peripheral apex. See the interaction the antagonistic interaction between *TFL* and *LEAFY; TFL* and *AP1* ensures inflorescence meristem identity in the center as well as floral meristem identity in the peripheral region.

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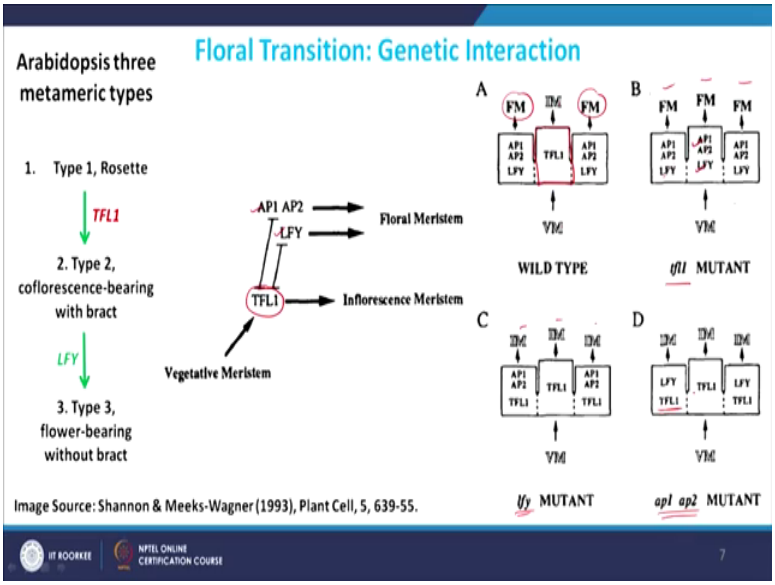

If you summarize this interaction, so during vegetative phase and the inflorescence meristem you have *TFL1* level very high. And then during floral meristem stage you have *LEAFY, AP1, AP2*. *AP2* is another A class of genes which you will see. If you look the transition from vegetative meristem to inflorescence meristem. In the inflorescence meristem you have expression of *TFL1, AP1* and *LEAFY* are in the peripheral meristem and this peripheral meristems are giving floral meristem.

What happens if you have *tfl1* mutants? So, since *TFL* is a negative regulator of *LEAFY*. So, if you do not have *TFL* here the *LEAFY* will start expressing here and *AP1* start expressing here. So, all three central and peripheral meristem all are going to take floral meristem identity.

On the other hand if you do not have *LEAFY* then *TFL* is going to expand its domain in the peripheral meristem and since you have high amount of *TFL1* in the peripheral domain basically it will provide inflorescence meristem identity. Similar kind of things you can look when you have double mutant of *AP1* and *AP2* here you have *TFL1* so it will be inflorescence meristem. But in the peripheral region you do not have *AP1* which means that *TFL* level will also go very high and *LEAFY* alone cannot specify full floral meristem identity. So, you will have essentially inflorescence meristem identity.

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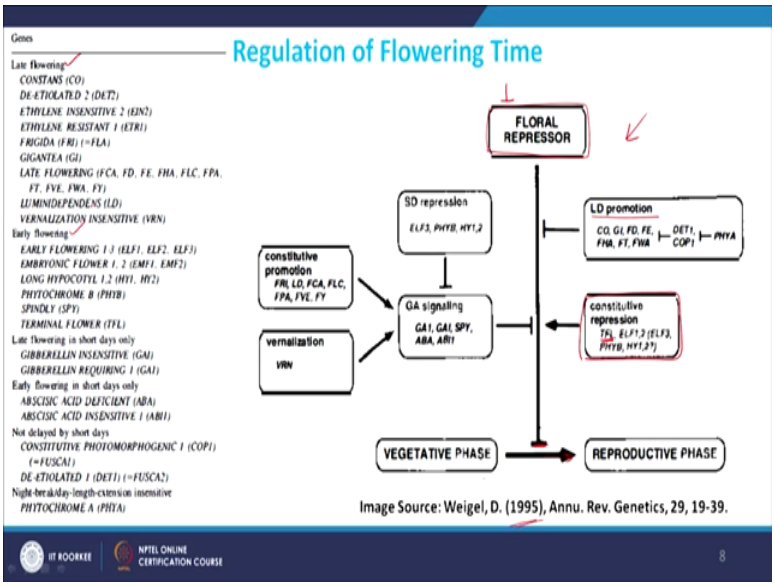

So, this regulatory network or this interaction is very crucial in giving a clear floral meristem identity at the peripheral region of the inflorescence meristem. But what are the genes which are basically deciding that what is the time when this identity should be given when this transition from vegetative to inflorescence meristem when the vegetative shoot apical meristem changes its fate and identity and becomes inflorescence meristem?

And definitely there is a floral repressor and it was hypothesized when it was given in 1995 when the plant developmental biology was still at the early stage and people have started looking very carefully plant developmental biology.

At that time it was hypothesized that there has to be a strong repressing signal during the vegetative phase and how to acquire the reproductive phase. One mechanism could be if

you repress the repressor, that will basically activate the flowering or if you start a new activator of the flowering. And when you look all the data, when you look the mutants identified or the genetic networks you will find that both pathways exists to ensure flowering at a particular time period.

There is a constitutive repression based mechanism and it has *TFL* in it; and they they strongly activate this process of the floral repression during the vegetative phase. But you have long day promotion, day length is very important in long day plants to ensure the flowering.

Then, apart from that you have some negative regulators you have gibberellic acid which is playing very important role in deciding the flowers. And more than that, if you look here there is a large number of genes has been identified. If you have mutation in that either they show late flowering phenotype, early flowering phenotype and there are condition if they can be in long day condition or short day condition.

So, if you look all these genes and try to understand the genetic pathways or the regulators which basically ensures this process there are the four major pathways which are important for regulating the transition.

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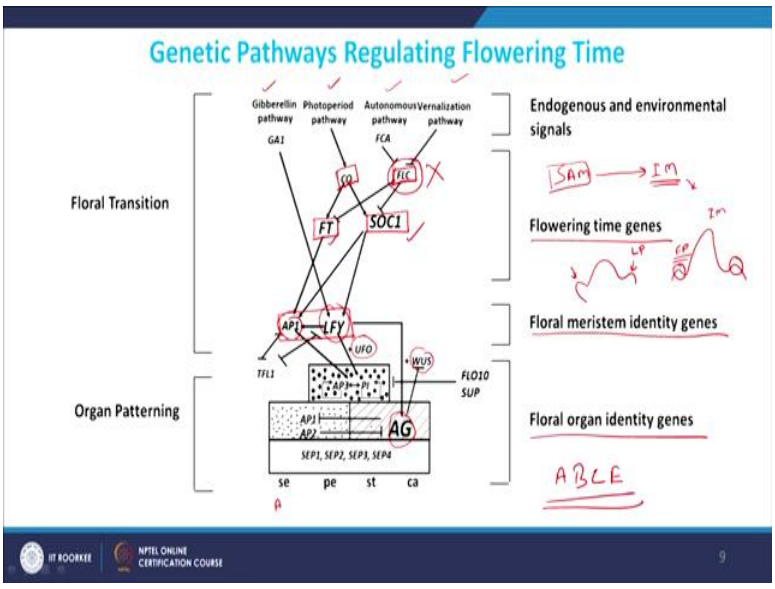

So, if you recall previous slides then you will realize that these two genes which are basically floral meristem identity genes they are very important *AP1* and *LEAFY*. So, one thing what you have to do you have to first ensure activation of *AP1* and *LEAFY* in the peripheral meristem to ensure a proper flower development the first step is this one.

So, whatever gene can activate *AP1* or *LEAFY1* they will basically promote the flowering. And the activity of *LEAFY*1 and *AP1* is regulated by two another important gene one is *FT* and second one is *SOC1*. And these genes are called flowering time genes and both are positive regulator of *AP1* and *LEAFY1*.

To ensure flowering, *FT* and *SOC1* has to be activated, but under normal condition or under vegetative growth condition both of this genes are kept repressed by a repressor which is *FLC*. *FLC* is basically negative regulator of *FT* and *SOC1*. And when there is a proper signal the repression of the *FLC* is repressed.

So, you have two pathway one is basically sensed by the gibberellic acid pathway where plant hormone gibberellic is very important in the activating flowering through this one. Gibberellic acid can activate *LEAFY* directly, but other pathway if you look the photoperiodic pathway they are positive regulator of *FT* and *SOC1* through *CO; CO* is *CONSTANT*.

When there is a suitable photoperiod this pathway will be activated and this pathway will activate expression of *CONSTANTS* and then *CONSTANTS* will go and activate *FT* and *SOC1*.

Another two pathways, one is the autonomous pathway and the other is vernalization pathway. These two pathways when suitable or in the condition to promote the flowering what they does they repress the *FLC* expression. So, *FLC* is a negative regulator of *FT* and *SOC1*. So, if you repress *FLC* essentially you are activating *FT* you are activating *SOC1*.

Arabidopsis thaliana is a long day plant. If day length is long day if all the autonomous signaling is at the right age, right strength and then vernalization signal is positive if everything is ok, then all this pathway will activate eventually *AP1* and *LEAFY* through *FT* and *SOC1*. And once *AP1* and *LEAFY* is activated, just now you have seen that then the meristem identity the meristem which is basically coming at the flank of inflorescence will take an identity of floral meristem.

So, there is the switch there is the change of the identity. During vegetative phase you have shoot apical meristem then after transition this shoot apical meristem changes the identity and it becomes inflorescence meristem; when it was shoot apical meristem then the peripheral primordia they were basically leaf primordia.

But when they become inflorescence meristem then, the peripheral primordia which is coming here; if this is inflorescence meristem it will become floral primordia. Which means that it has to activate some floral primordia specific genes and some of the genes which are very important in regulating the floral organ patterning. If it is floral primordia it is going to make flower and develop flower specific organs and flower specific organs are sepal, petals, stamen and carpals. And these job is done by a class of genes which is called A B C and E.

These class of genes are called floral organ identity genes. They provide a proper identity to each organs in the development. And all these genes are basically getting activated later on by *LEAFY* and *AP1*. So, if you look *AP1* is getting activated by *LEAFY*. *AP1* is dual function gene, so it is floral meristem identity gene at the same time it is also A class gene which is responsible for sepal development which you can see in the later slide.

So, basically *LEAFY* activate *AP1*, A class gene. *LEAFY* together with *UFO* activate B class gene which is *AP3* and *PI* in *Arabidopsis* and *LEAFY* together with *WUSCHEL* activate *AGAMOUS*, *AG* gene which is basically C class genes. And then you have another set of genes which are called E class gene.

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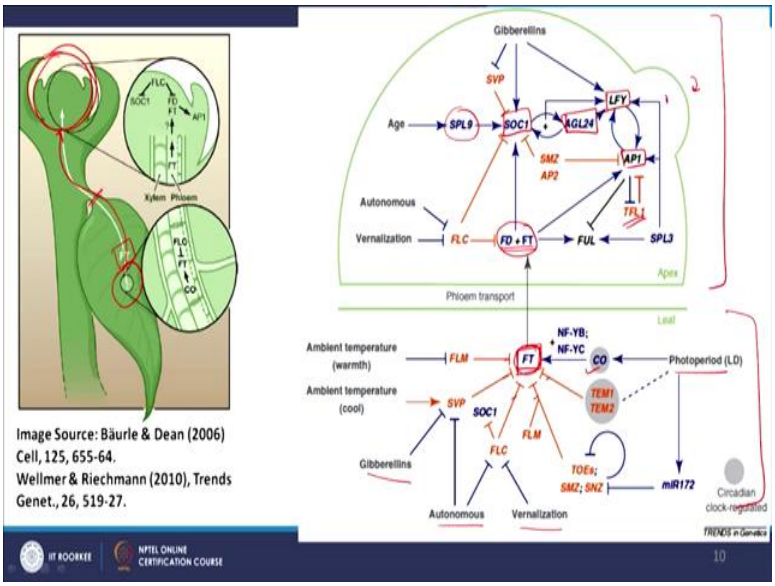

Another important thing to mention here that the most of the signals are getting received in the leaf and transition of the flowering or the decision to change the meristem fate occurs at the apex. The question is that how this signal which is being received in the leaves are getting communicated to the apex? And there is a long distance signaling

involved here and one important molecule which is *FT* which is a mobile florigen and moves from leaf to the apex.

So, *FT* is getting activated in the leaf and then it moves through the vascular tissue precisely through the phloem and when it reaches to the apex in apex it activates the genes. So, if you look here this diagram, so this is your leaf and this is your apex. So, in leaf *FT* is kept repressed by several mechanism during vegetative phase.

In leaves there are multiple mechanism which are repressing *FT*, but when there is a right signal and photoperiod it activates *CONSTANT* and *CONSTANT* is activating *FT*. And when you have *FT* and other pathway like vernalization pathway, autonomous pathway, gibberellic acid pathway, they are also working to activate the *FT*, but they are repressing the repressors.

So, eventually if you look here *FT* is getting activated in leaf and then it is moving through the phloem to the shoot apex once it reached to the shoot apex it interact with another gene called *FD*. And they in the apex in the center of the meristem they activate *SOC1*, they activate *AGAMOUS LIKE 24* and the interaction between *SOC1* and *AGAMOUS LIKE 24* along with *SPL*9 genes which are very important in maintaining inflorescence meristem in the center.

But at the flank region or at the peripheral region these pathway or these mechanisms they activate *LEAFY*, they activate *AP1* and then *LEAFY* and *AP1* or interaction between *LEAFY1* and *AP1*; and then repression of *TFL* in this region essentially ensures floral meristem identity to this peripheral region.

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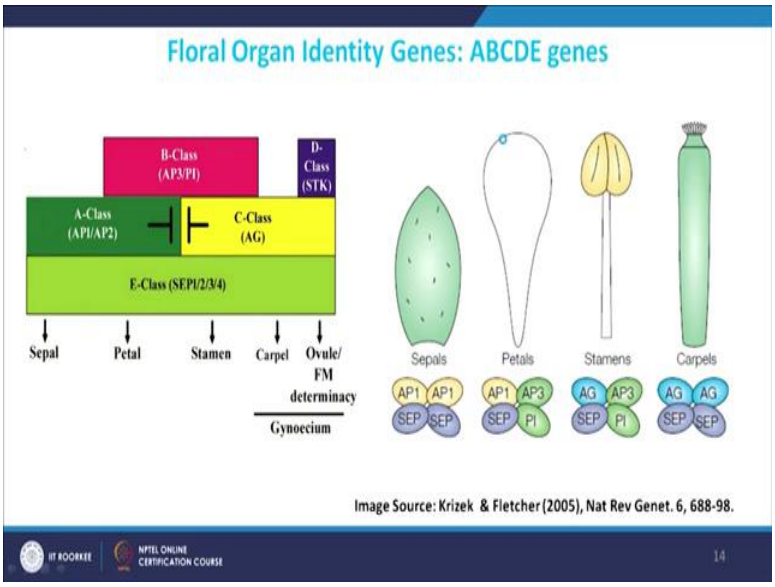

So, there are three classes of genes A, B and C and how they express? So, they express in the overlapping manner.

So, A class gene they get activated in the sepal and they continue in the sepal and petal primordia. B class genes get activated from petal and it is in petal and stamen primordia. And C class genes get activated here in the stamens and carpel primordia. Whereas, E class genes are expressed everywhere. So, these genes are very specifically getting activated and *LEAFY* is involved in activating these genes in a very specific domain.

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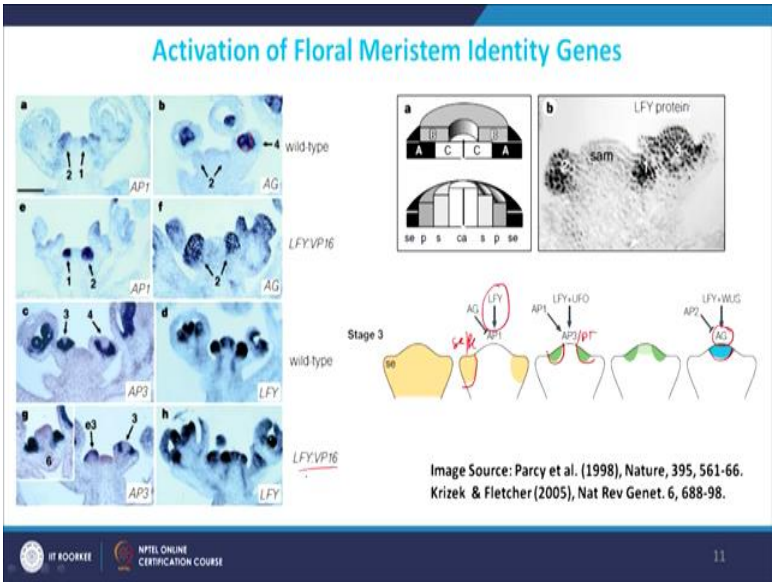

LEAFY activate *AP1* in the region of the meristem which is responsible for which is basically sepal and petal zone. Then it activates B class gene which is *AP3* and *PI* in petal and stamen zones and then it activates, finally, C class gene which is *AGAMOUS* in the carpel zones.

So, function of *LEAFY* is not only to give a meristem floral meristem identity, but also to ensure that downstream processes of floral organ patterning is also getting activated in a very specific manner or in a very domain specific manner. The expression pattern of *AP1* you can see in the developing sepal and petal primordia; if you look *AGAMOUS* it is only in the center. And if you look *AP3* it is basically in the domain where you have petal and stamen primordia. And you when you over express *LEAFY 1* you can see that expression domain of all these genes are getting expanded.

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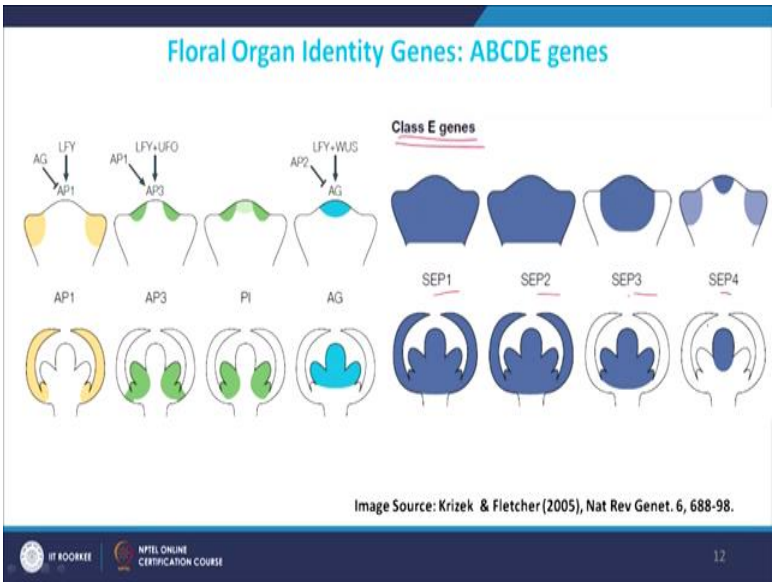

So, this is just expression pattern of these genes at early stage and when there is a flowering. E class gene is very interesting in a way that it works like a cofactor for A, B, C class genes. And there are four E class gene *SEPALLATA1, 2, 3* and *4* and if you look the expression pattern, *SEPALLATA1* and *2* is quiet broadly expressed, but *SEPALLATA3* and *4* basically expression is restricted.

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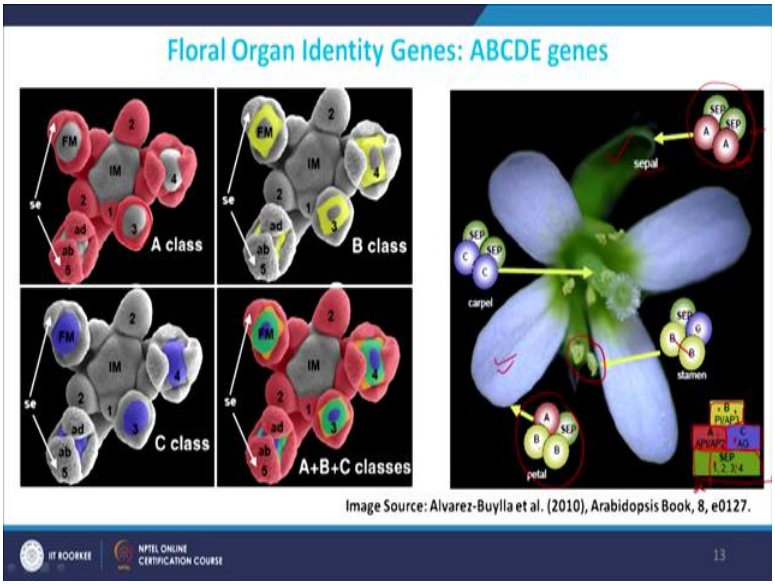

And how they regulate the organ? This is overview of the expression pattern A class gene in the sepal B class gene you have in the petals and stamens and then C class genes you have in the center this is A plus B plus C class gene. So, you can have this kind of specific and overlapping expression pattern and this is very important because their interaction their overlap basically defines the identity.

In sepal you have only A class gene and E class gene. So, A class gene is here B class gene is here C class genes are here E class genes are here. So, if you look this region only this region which is going to make sepal, it has only A class gene and E class gene and this interaction basically gives sepal identity.

In petals you have B class gene A class gene and E class gene. So, you have A B and E and these three genes together ensures petal identity.

Similarly, in the stamens you have B C and E and this gives stamen identity. And in very center where you have the carpel you have only C and E together ensuring carpel identity in sepal you have A, E interacting in petal you have A, B, E interacting.

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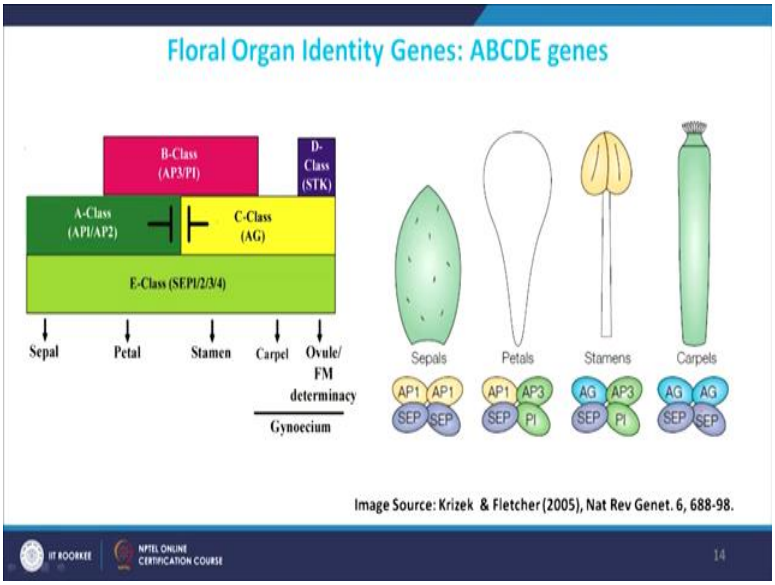

In stamens you have B, C, E interacting and in carpel you have C and E interacting.

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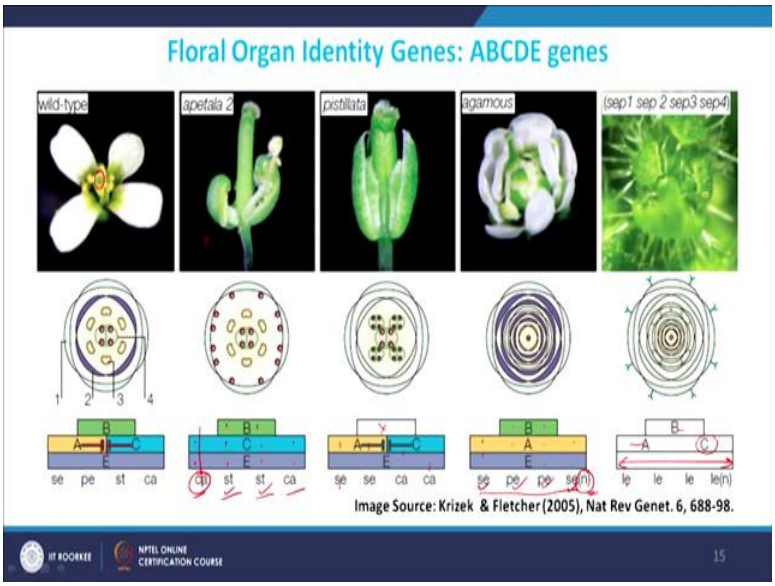

And this you can see here if you have mutant in this what happens.

Another important thing here is that A basically restricts the domain of C and they are mutually antagonistic. So, they are restricting their domain. If you have A class mutant what happens that the restriction of C disappears and C can continue expressing here. So, this region is now having C and E and C and E will give carpel then you have B, C, E it will give stamen B, C, E it will give stamen and here you have carpel. So, if you have A class mutant you can clearly see that your flower will have carpel, stamen, stamen, carpel.

Similarly if you do not have B class gene, then you are going to have sepal here sepal here, carpel, carpel. This you can see here you have this two whorls of the sepal and then two whorls of the carpel.

Third important thing if you have C class mutant gene what happens that A class gene will now continue expressing here. So, here you have A and E it will give sepal A, B, E it will give petal A, B, E it will give petal and here you have only A and E it will give sepal but another important thing here what happens that these structure basically continued.

So, there is a flower within flower phenotype which tells that in this mutants the floral determinacy is lost. So, normally what happens in the wild type, when your final organ which is carpel, when carpel is made the organogenesis is stopped and that is called the state of determinacy, but C is ensuring that determinacy if you do not have C class genes. So, apart from the floral organ defect pattern, but you also see a loss of floral

determinacy but important thing if you do not have E mutant here E gene; so, basically *SEPALLATA 1 2 3 4*.

So, if you have mutant for all of them although A genes B genes and C genes are fine, but they cannot function which means that their activity is dependent on E class gene and in this case you can see that all the whorls are going to have leaf like structure.

So, this is clear that the activity and interaction between A, B, C and E is important for regulating floral identity.

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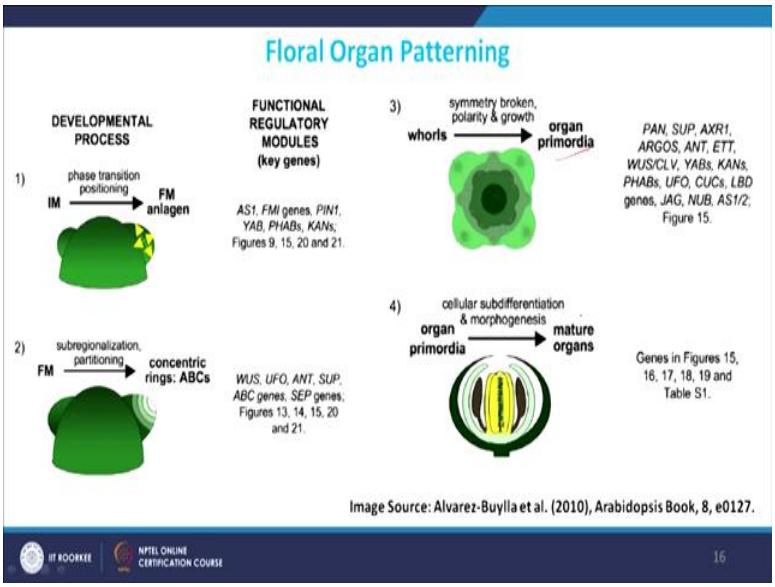

So, these are different organ patterning's. Inflorescence to floral meristem these are the different steps. Then in the floral meristem, you have the concentric ring formation sepal, petal, stamen and carpel. Then at the same time here there is symmetry broken, you have organ growth and then maturation of the organ. So, these are different stages of the flower development and then you have different genes which are regulating different stages.

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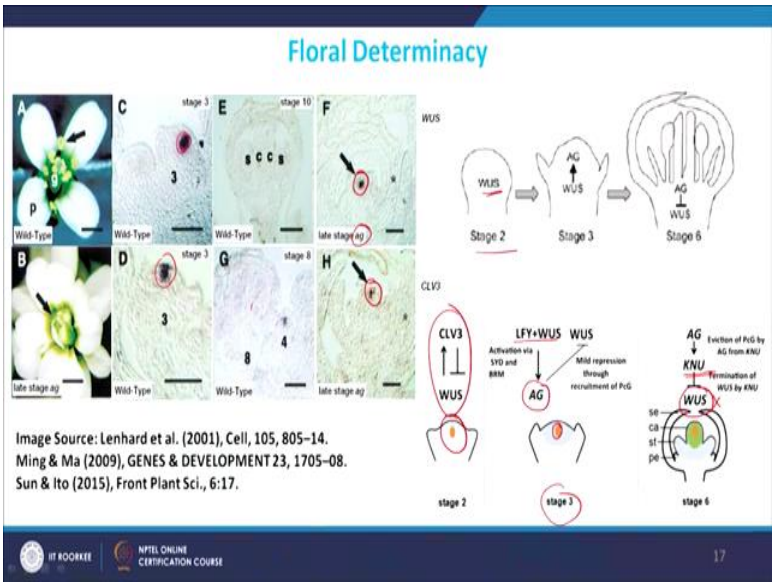

The final thing which is important is the floral determinacy. As I said that flowers are determinate in nature. So, they terminate once final organ which is carpel is made and you see here if you look the expression of *WUSCHEL* which you have already studied in previous classes that *WUSCHEL* and *CLAVATA* they are very important regulator of this stem cell activity.

And in flower what happens that at early stage there is a strong *WUSCHEL* expression and strong *CLAVATA3* expression. But once the flower is mature you can see that *WUSCHEL* and *CLAVATA3* expression is almost disappeared, but if you have *agamous* mutant which is basically C class gene mutants where you have the organ defect as well as determinacy defect.

You can see even at the late stage, the expression of *WUSCHEL* and *CLAVATA*3 are maintained which suggest that the determinacy is lost because there is no stem cell termination mechanism. So, under normal condition what happens at early stage you have *WUSCHEL* and *CLAVATA* on. At this stage if you look *WUSCHEL* and *CLAVATA* and they are basically maintaining stem cell pool in the center and that is why there is an indeterminate growth.

But at the later stage when this flower is in the process of organogenesis, *LEAFY* and *WUSCHEL* together activates *AGAMOUS* in the center and this *AGAMOUS* is responsible for carpel and stamen identity. But at the same time at the later stage when the identity of carpel is already established this *WUSCHEL* activate gene called *KNU* and this gene basically goes and repress the *WUSCHEL* and this is important factor. Once

you replace the *WUSCHEL* means there is no *CLAVATA* once the *WUSCHEL-CLAVATA* signaling pathway is lost, then the center region cannot maintain the stem cell niche and that is why the growth terminates.

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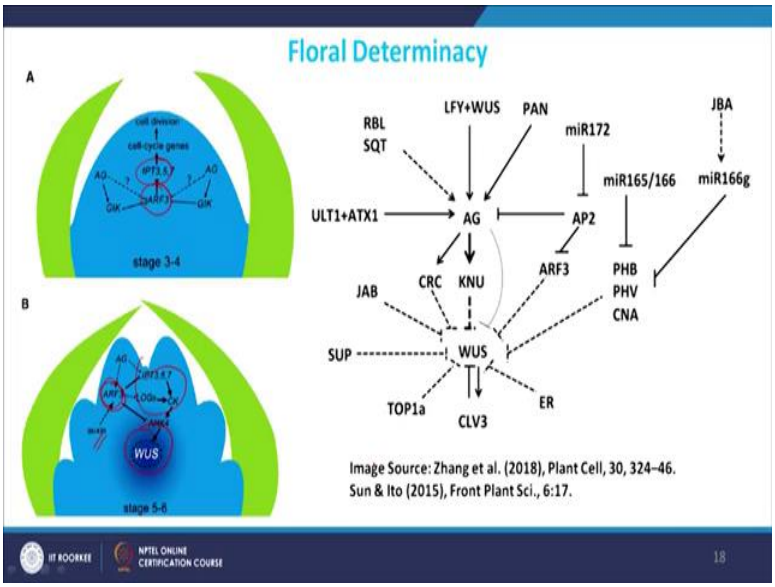

Apart from the *WUSCHEL* and *CLAVATA* cytokinin is also playing important role in maintaining the stem cell pools; and you know that this is the biosynthesis enzymes cytokinin is having a positive role on the stem cell maintenance.

At early stage Auxin response factors, *ARF3* is kept repressed, but at the later stage this *ARF3* is getting activated by Auxin. Auxin is very specifically getting activated in the carpel at the later stage.

So, carpel has Auxin once Auxin is there in the carpel it activate *ARF3*, *ARF3* goes and repress the cytokinin signaling pathway and eventually that repress the *WUSCHEL* expression domain. So, this complex mechanism all this regulators together ensures that, at the end of floral organ patterning the meristems are getting consumed up and there is no mechanism to maintain the dividing cells.

Thank you very much we will stop here and in next class we will discuss about the cell-cell communication in plants.

Thank you.