Introduction to Developmental Biology
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Lecture No – 23
Plant Development (Part 3 of 3)

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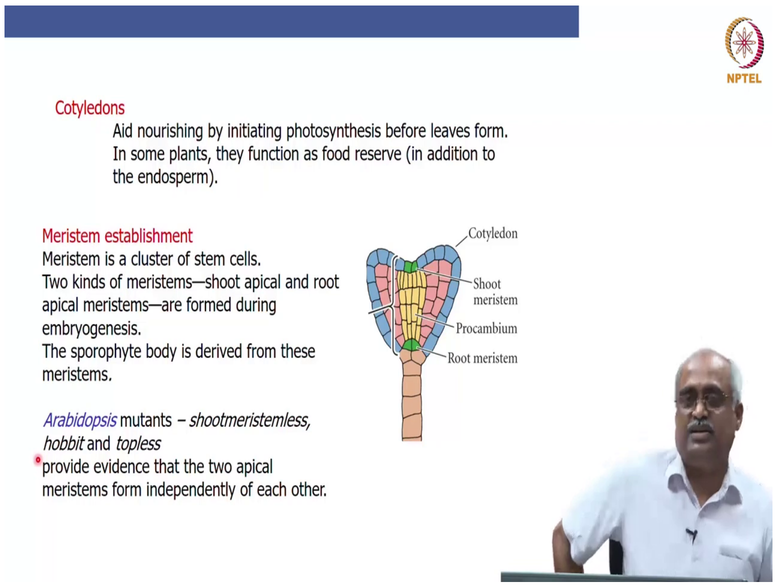

So students welcome back to the developmental biology course. In the last class, we were discussing the early embryogenesis implants, specifically, we focused on the body plan establishment. That is, the radial patterning that happens at the globular stage of the embryo and then the apical basal axis establishment that gives rise to the shoot and root in a primordium. So today, we continue from there. So I told you that from globular to the next stage that is heart shaped, the transition is made obvious by the formation of the cotyledons.

When the cotyledons start to, you know, form then the global shape changes into heart shape because of these two protrusions. And by that stage I told you that the apical basal polarity becomes obvious and one of the three purposes of embryonic development, that is, setting up the apical meristems that is the shoot apical meristem and then this is the root apical meristem. So, these two become quite obvious by the time of heart shape.

So now we will go to the meristem establishment in a minute, but before that let us consider cotyledons briefly. So these are the early leaflet structures that form when the seed is going to germinate and these are involved in a very initial photosynthesis and thereby establish the new food production when it is going to germinate. And in addition to initiating photosynthesis, cotyledons store food, you know, carbohydrate lipids and proteins and therefore they provide additional nourishment in addition to what is provided by endosperm. And in some of the plants these cotyledons also facilitate the food reserve transfer from the endosperm to the embryo. So that is about the cotyledons. The cotyledons form not from any one specific layer it is from the embryonic tissue in general.

So the next we are going to look at are the meristem establishment. So meristems are nothing but a cluster of undifferentiated cells equal into what we call stem cells in animals and these are clusters that are there on the or top that is the in the apical basal parallel polarity on the apical side and that is the shoot apical meristem and then this is the root meristem.

So these two are produced more or less independent of each other. And the entire sporophyte body is derived from them. For example, the branches or leaves and finally the reproductive organs inflorescence flowers all come from the shoot apical meristem. And similarly the root cap and the root tissues they all come from the root meristem. So the entire sporophyte body are derived from these two meristems that are set apart during the embryogenesis.

And genetic mutants isolated in arabidopsis indicate or provide evidence that these two apical meristems form independent of each other. For example, there are mutants like shoot meristemless gene, you know, mutant alleles of this gene and they do not produce the shoot meristem but in them the root meristem formation is unaffected. So similarly in hobbit the opposite happens like the shoot meristem forms properly but the root meristem does not form.

And in topless mutants you have the shoot typical meristem like cells instead of forming root tissues. So these are the mutants that provide, you know, a handle for us to get to the genetic control and therefore understanding the molecules involved in these early body patterning in the plants. So these are still emerging and a very active area of research.

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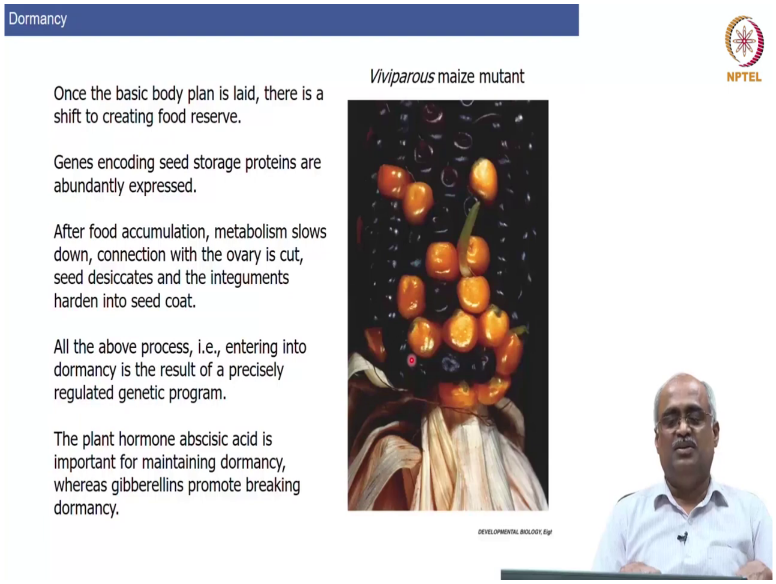

So once the body patterning is established like right now we are in you know if you want to draw parallel with drosophila it is like segmentation of the embryo is done. So right after that there what will happen it will go into forming the other tissues and organs and like that it will move on in the development. But that does not happen in plants instead, the plant development takes a break at this step. Once the body planning is established, that is the radial and axial patterning is established. The next thing, what it does is it starts to produce food reserves.

So in the last class I mentioned that so here the embryo needs to move from the parent, the sporophyte, to other locations where you know they can have a conducive environment and food reserve to grow up. So the seed dispersal is an essential part of how plants reproduce and, you know, develop. That necessitates a requirement of a break at this step in the development and instead genes involved in making storage reserves they get activated.

For example, seed storage proteins are abundantly expressed and then the food acculation starts instead of continuing with the plant development embryonic development. Now, the focus is on expressing the food reserve related genetic activity and it accumulates lot of food. Once that is done, then it takes another break - so that break is what we call as dormancy. So the metabolism slows down connection with the ovary is now cut like the placental connection is cut and the integrands start to harden and become a seed coat and the embryo proper or the endosperm or the seed loses a lot of water, it desiccates.

Basically everything becomes very compact packed food reserve and then the embryo alone and all are protected by the seed coat, which is basically integument that hardens into a seed coat. And obviously all of this process is quite complicated and have to happen in a certain order and therefore it is all very well and precisely controlled by a genetic pathway which is still being elucidated. And an important point to note here is the plant hormones control these genetic pathways that regulate dormancy.

So for example, abscisic acid is a predominant plant hormone involved in dormancy like entry into and maintaining dormancy and gibberellins do the opposite. So these promote breaking dormancy which will you know discuss in the next slide. But suppose if you have a problem in this genetic pathway for example, the viviparis mutants of maize. So what happens is instead of these seeds the kernels staying in the ear in dormant stage they continue to the next step and they start germinating.

So that is not going to be a successful reproductive strategy. It is not going to have access to, you know, the minerals and other nutrients from the ground and taking up water and establishing as an independent individual plant is not going to be possible if this happens. So that is why the entry into dormancy and then subsequent seed dispersal is very critical. So therefore there is a lot of interest in understanding how the dormancy is regulated.

So next we will look at, so once dormancy is induced when do they actually come out of dormancy and then continue the development.

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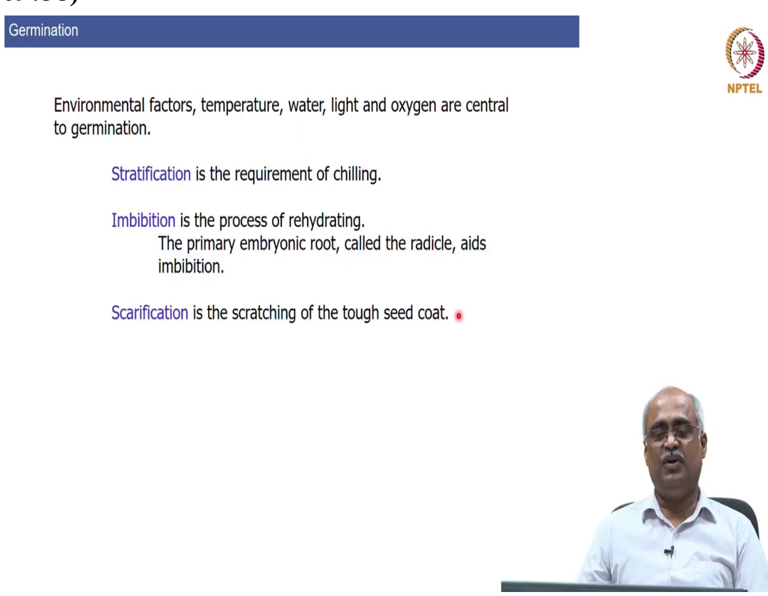

So that process we call as germination and germination as you can imagine, should happen at the right time in the right environment. You know, like plants cannot, the seed cannot germinate and make a successful plant by germinating in the middle of deep winter. If it is, you know, in a temperate geographical area where it is frozen for during the winter period. So it will be unsuccessful and the seedling that germinates will die.

So therefore the environmental factors such as temperature, water and light, you know is there sufficient light and is there water availability and is there enough oxygen all of them are central to determining germination. So, for example, the long day, short day pattern matters with respect to light and temperature and so on. So again therefore the environmental cues are sensed quite efficiently by the dormant seed and only under the appropriate environmental condition it is going to start the germination.

Here are some of the examples of such factors one is stratification or you know like another word for similar thing is vernalization at a different stage of development we use the word vernalization but here, for the germination we use the word stratification, which is nothing but having experienced the winter. That is, the seed needs to necessarily be at a colder temperature for a certain period of time to be able to germinate that sort of indicates that the seed experienced winter. Now the winter is over and the spring is coming so therefore it is appropriate time to initiate germination.

So the experiencing of the cold temperature is a prerequisite in those species for germination and that we call as stratification. So in some situations where the plant has to experience similar cold temperature before inducing flower formation there we call vernalization. And second, there should be enough water so that remember in dormancy the seed lost a lot of water it desiccated now for the embryo to grow up it has to take up water and that process we call as imbibition.

And imbibition is facilitated by the you know the embryonic root called the radical. So once the radical forms, it helps in taking up water and the embryo gets rehydrated and then it is ready to activate metabolism and to move on with the development. And in some cases where the seed dispersal is really key, the seeds really need to experience really harsh conditions. For example, some of the seeds really need to be, the thick outer coat, needs to be injured or rather scratched and that process is called this scarification.

For example, when the seed is eaten by a fruit eating organism like frugivore and in its gut it experiences acidic conditions. And that helps in this scarification and that is a prerequisite for germination. So that sort of a thing, like having gone through the gut indicates that it has been disposed as well. So the frugivores in that way help in dispersal as well as to initiate germination. So like this the main emphasis here is all the environmental cues are really crucial in determining the germination.

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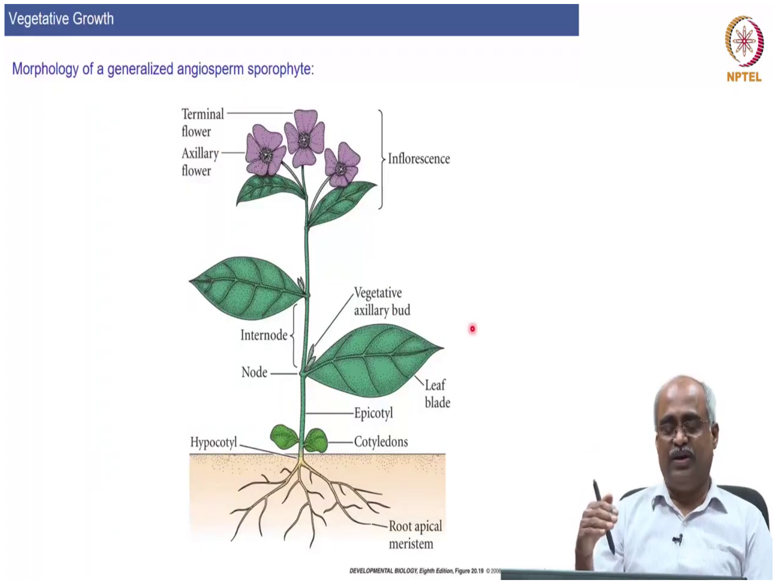

So next what we are going to do is fine, so the after the initial body patterning it started accumulating food then it slowed metabolism desiccated, cut the contact with the sporophyte and then desiccated and became dormant, hen under appropriate conditions it is germinating. So now we will get back to the development and see how the initial development happens. So given that this is an introductory developmental biology course we are not going to look at all aspects of plant development.

So we are going to choose you know some processes that are very well understood genetically and we are going to focus only on that. So primarily we are going to focus on flower development. So before going into that I will briefly introduce the structure of a typical angiosperm ,that is, a typical flowering plant, the sporophyte, you know, generation of angiosperm. So shown in this cartoon is one such flowering plant with the flowers there.

So you have the root and then below the cotyl, we call the hypocotyl then you have the cotyledons then cotyledons to the first leaf we call the epicotyl, then you have the leaves. Then you have the axillary bud, that is formed between the stem and the leaf at that angle and then you have the internode then you have finally flowers. So flowers can be axillary or terminal flowers. And a stem like structure having only flowers we call that as inflorescence. So this is morphology of a generalized angiosperm sporophyte.

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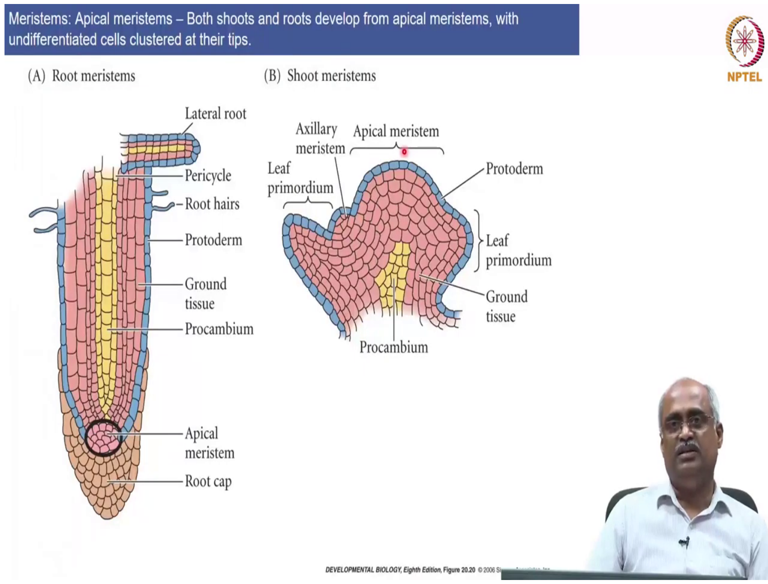

So now, let us get to development. So the first thing is, we are going to focus again on the two meristems, so what do they do, what kind of structures they make up and then we are going to quickly jump into flower development. So the rest of leaf development stem development, vegetative growth, all of that we are going to kind of pass through. So this is a root and this is the root apical in the meristem area and these are the cluster of meristematic cells. They give rise to this root cap.

So these cells lubricate and help in penetrating into the soil when these upper regions elongate and divide and when they push this apical meristem down. And these as they are shearing against the soil, these are going to be lost anyway, and they are going to be newly made again and again by these meristems that will differentiate. So these meristems, meristematic cells, are like animal stem cells in the sense that they divide produce one daughter cell that is differentiating cell for example making root cap another one is a meristem itself.

So that is how the mitotically dividing undifferentiated population of cells are maintained here. So in addition to the root cap, the root apical meristem cells give rise to the other three layers of the root as well. Like remember, I told in the last class, protoderm, the dermal tissue then the ground tissue from which pith and other cortex etc are formed and then the procambium from which the vascular tissues are going to form. So in addition, the growing root produces lateral roots and therefore the lateral meristems set up also.

They come from, they originate from the innermost part or that is what we call as pericycle. So this is from the cambium. So these cells give rise to the lateral root meristems, and that is how the root branches form. So now let us look at the shoot apical meristem. Again similarly, it is required for generating all the, you know, shoot organs like leaves, stem, branches, and eventually producing inflorescence and flowers all of that come from this. And in addition and the shoot apical meristem also gives raise to the axillary meristem.

And the axillary meristem unlike the root lateral meristem which comes from the innermost layer, this comes from the surface layer of cells. So they give rise to the axillary meristem. And, so that is essential for branching as well as the leaf primordium to start like for example here you have a leaf primordium developing and here another one coming out and in turn it makes the same three layers of cells as well.

And the root, in the apical meristem the shoot apical meristem, the genetic studies as well as the chimera experiment, that is transplanting a part of a layer of meristematic cells of one genotype to another genotype and observing what structures it contributes too. So that is what a chimeric experiment is like, you are taking analogous tissue from one genotype and then you are grafting onto a similar location on another any plant that is of a different genotype.

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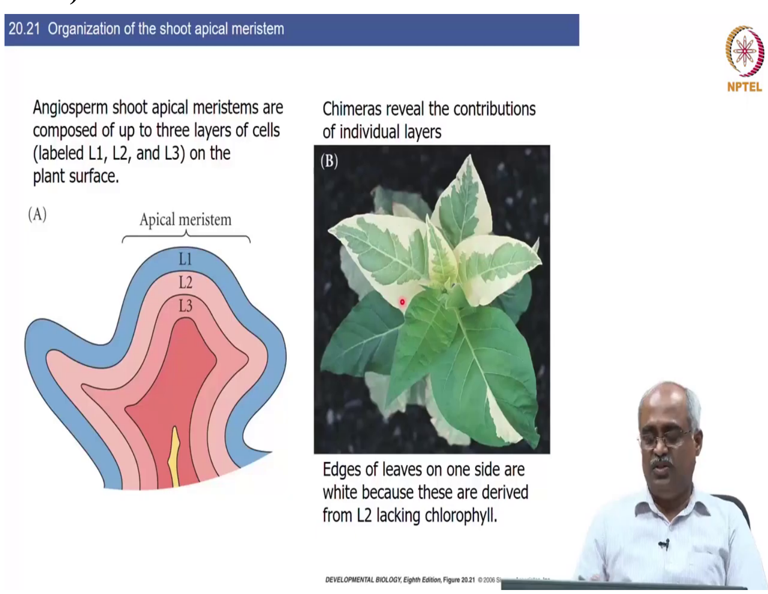

So those experiments helped people identify three main layers of the apical meristems here labeled as L1, L2, L3 and these layers give rise to different structures so that is what people have understood by doing these kinds of experiments. For example, here in this figure on the right side you see some variegated leaves while then other leaves are properly normal fully green. And that is because in the initial you know the apical meristem of this plant on one side of it where the L2 layer has been transplanted from a genotype that is defective in producing chlorophyll.

So therefore the L2 in this particular case does not produce chlorophyll. L2 is the layer from which the edges of the leaves form so as a result here the edges look white. And the L1 normally does not, you know, produce chlorophyll and L3 does not contribute to the edges and that is why the middle region coming from L3 has the chlorophyll. So this is how the contributions of the different layers have been understood.

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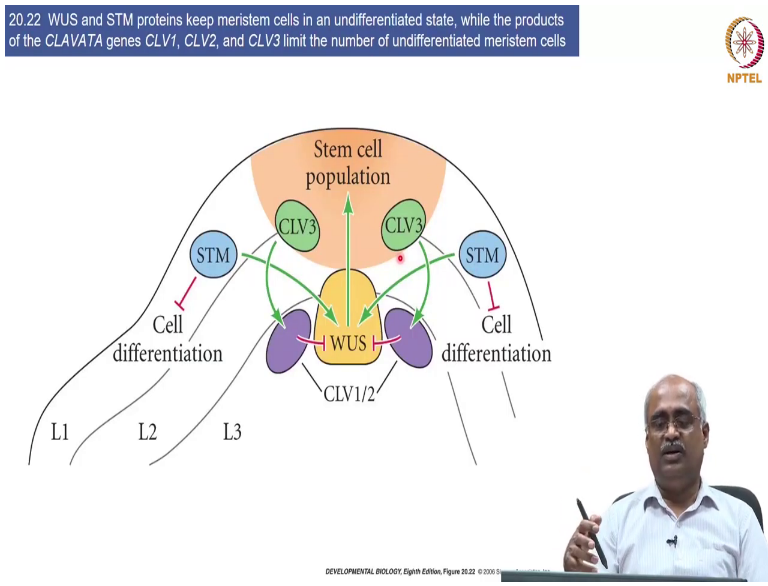

So now what we are going to look at is how these meristematic cell populations are controlled. So this is a very important aspect of any stem cell system, particularly adult stem cell systems where maintaining a balance between the number of cells that are in the undifferentiated state that is stem cell fate versus the number of cells that are differentiating need to be balanced. If you do not balance this then you will have two, like suppose if the self renewal if that is prominent then the organs will not have proper contribution of differentiated cells and they will not form properly.

The right proportions will not be there. And in an extreme case if there is no differentiation then you will only have a mitotically proliferating tumor like a lump of cells and no tissues will be formed. And if there are no stem cells maintained at the other extreme, like everything, all cells, differentiate then there will be no more stem cells to give rise to further development like no new organs or new kinds of tissues can be formed. So therefore the size of the stem cell population needs to be tightly controlled and that has been understood to some extent through genetic studies in arabidopsis and that is what is cartooned here.

So essentially, the gene wuschel promotes the cells that produce wuschel, promote stem cell fate on the layer above it and that makes them remain as stem cells. But wuschel activity is not uncontrolled, it is controlled by a group of genes called clavata genes. So for the spelling you can see the heading here, clavata genes. so the clavata genes antagonize wuschel, so clavata genes are 2 and 3 together form a receptor, a serene therion kinase enzyme and a receptor that is what those 2 encode.

And they receive the clavata 3 signal so this is like a ligand produced by the cells that are actually in proliferative fate and they suppress these. So it is kind of a feedback, negative feedback and that ensures that this population is, you know, at the normal level. So it restricts the size of this. So if you do not have clavata then wuschel will not be negatively regulated and the stem cell population will enlarge and as a result you produce a more number of organs which we will see in the next slide which is a clavata mutant.

So that is what will happen. And in addition to this clavata and wuschel regulation, you have another gene called STM which inhibits differentiation and in addition it also promotes the wuschel expression. So this again helps in making sure that these three genes put together make sure the population of meristem is maintained at a normal level. So this is how the self-renewal population and the cells that are differentiating is balanced.

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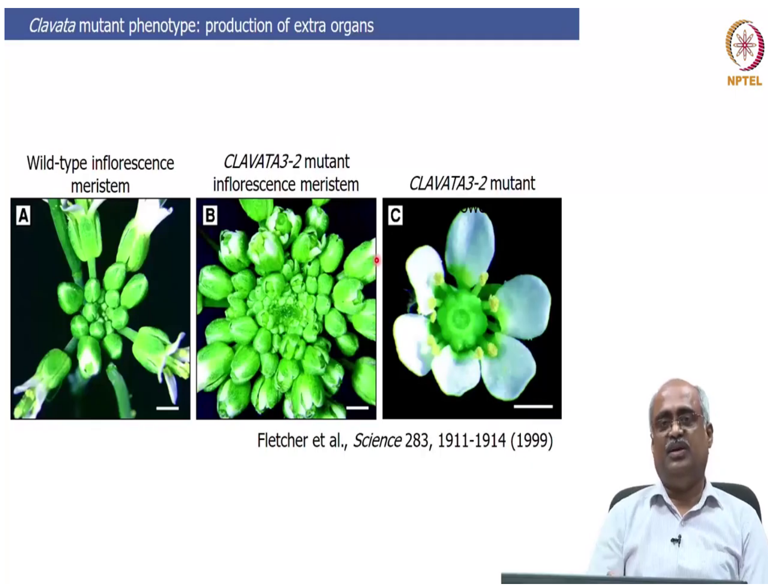

So here you have three photographs showing, you know, what happens when you have clavata mutation. So this is the wild type where you have normal number of flower buds forming and these are the flowers that are coming up and this is a clavata 3, 2 mutant. We are looking at the meristem and the in fluorescence meristem and then you can see there are a large number of flowers forming It is due to bigger size of the inflorescence meristem.

So as a result it is giving rise to more floral meristems and therefore more flowers are forming. And here we are looking at the defect in floral meristem itself. We will learn in detail about inflorescence minister floral meristem etc. in another few minutes. Here we are looking at another meristem defect. So here we saw an inflorescence meristem: what happens if it is big? If it is big it gives rise to many more floral meristems and as a result you get many more flowers.

And in the floral meristem when you have, when its size is big due to clavata 3, 2 mutation then you have more floral organs produced. Inflorescence meristem defect bigger meaning more flowers and when you have bigger floral meristem meaning more floral organs, like for example you see here it has 6 petals while normally it is only five, and similarly if you look at the you know the stamen you have again a lot more numbers and again if you look at carpel there again they are more than adult.

So they should have been 5 each and instead they are all more of that. So this sort of highlights the importance of regulating the self-renewal differentiating population to make an optimum body plan that is optimum for generating reproductive structures and seeds and so on.

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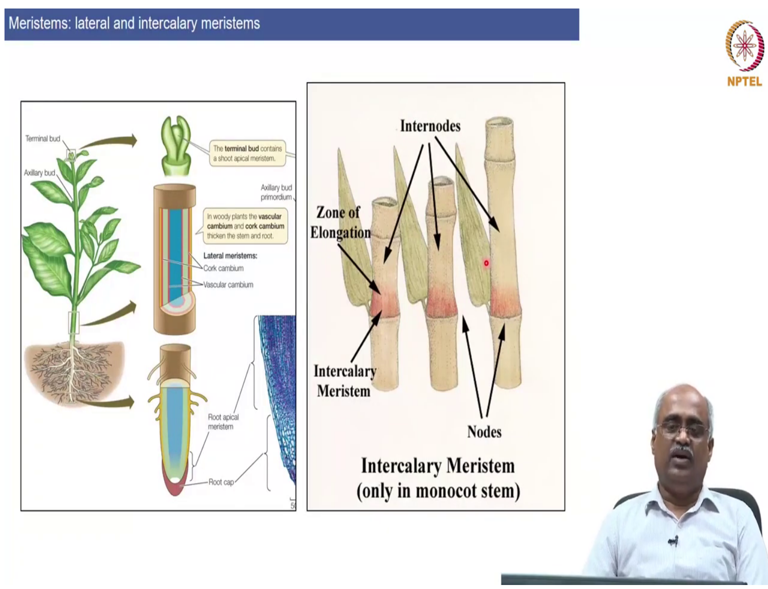

So this is about the, you know, regulation of the shoot apical meristem and we also discussed the root apical meristem in addition there are other meristems to other meristems as well we will briefly understand what they are and then we will move to the flower. So, if you take a cross section of the shoot, the stem portion, and if you look at it so you have these red, the two red layers of cells. So these are lateral meristems. So these give rise to cells that help, in broadening, like growing in a broader way like the thickness of the stem increases or the girth of the stem, you know, radial expansion of the stem increases.

And that radial expansion of the stem is contributed by the cells arising from these two meristems the cork meristem the that is the cork cambium, that is the outer one and then you have the vascular cambium that is inside and these two meristematic cells are called the lateral meristems and they help in the, you know, the increase in the width of the stem. So that is you know another set of meristems that work in the plants and contribute to growth and this is primarily in the dicots.

And monocots have additional meristem distributed differently and contributing in a different manner. So instead of having continuous inner layers, lateral meristems so they are present or dispersed within the adult cells and these are called the inter-calorie meristem. So this is like the node and just next to the node you have these intercalary meristems dispersed among the adult cells like differentiated cells. And these meristems divide and elongate, giving rise to the plant growing you know in the apical basal direction that is, the shoot grows top to the top.

The plant becomes taller due to that sort of intercalary meristem driven growth. So this is quite common among the monocots. So we have learnt about shoot apical meristem, root apical meristem and then we saw what kind of tissues come from them and now we have learnt about the intercalary meristem as well as lateral meristems they respectively work in monocots and dicots.

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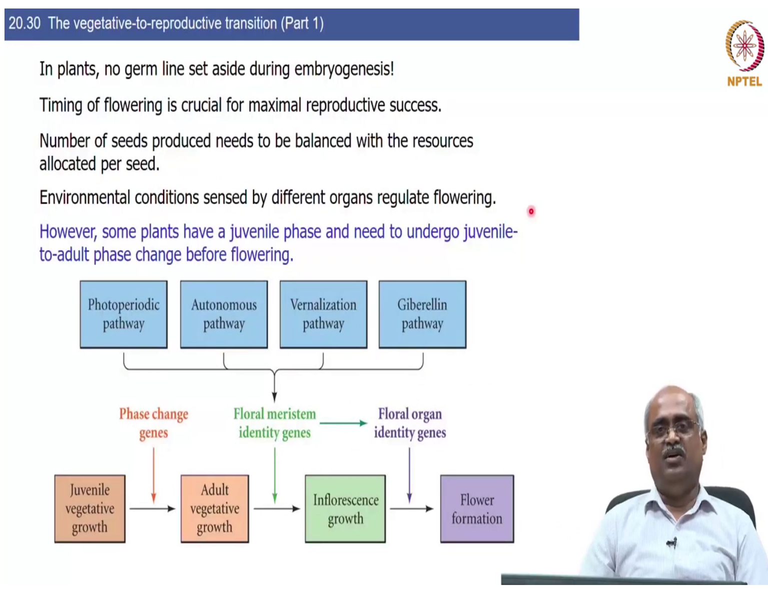

All right, so now I am going to skip how the leaves form and how the vegetative growth happens and develops instead what we are going to do is we assume the plant has grown to the adult stage and now how the reproduction starts? So, an important point to note here, which I described or briefly mentioned at the very beginning as well in the previous class is that the plants do not make germ cells. They do not set apart the germ line during embryogenesis, so instead the cells that can undergo meiosis, they are derived from these meristematic cells, the shoot apical meristem when it switches from vegetative to reproductive phase.

And that again, like how I was emphasizing on how the environment matters for germination. So similarly, here again the environment has to be sensed and appropriate environment required for optimum reproduction is essential. So merely switching to making reproductive structures under harsh conditions, you know which is not conducive for setting the seed production is not going to be suitable for optimum reproductive capacity.

So timing matters. Timing of flowers is crucial, is it going to flower in the deep winter or it is going to do in the spring or is it going to do in the transition from summer to winter. So when flowering will be optimum for that given species, so that has to be sensed. So obviously, photoperiodism, that is the light duration, you know, is it a short day or long day sensing that is going to be crucial in transitioning from vegetative to reproductive.

So first of all, I want to emphasize the point that there is a transition so during the vegetative growth the apical meristem keeps producing leaves branches, leaves branches, leaves branches, that is it. So the fate of those meristem cells need to transition and now it should start making reproductive structures. For example, instead of producing leaves and stems it should now produce inflorescence and the inflorescence in turn should start producing, you know, flowers and that is what we call as the vegetative to reproductive transition.

So such a transition exists and that is regulated by a variety of environmental conditions and I have just alluded to the importance of the light. And second, the number of seeds produced need to be balanced with the resources allocated per seed. So are you going to provide a seed a lot more than the food reserve it needs? Then you will end up producing only fewer seeds and that may not be evolutionary successful. So therefore an optimum is determined. So this is the food reserve available with me as an adult plant entering into vegetative reproduction.

With this, this is the maximum seed that can successfully, you know, go and germinate that can be produced. So that optimum needs to be arrived at and these are all tightly genetically controlled by sensing the environment. So, and these environmental conditions vary. Not all angiosperms sense the same environment and induce a reproduction. It varies, you know, some flower before winter like from for example apples and other berries and some of them flower like, for example neem trees in India they flower in the spring right after the winter season just before summer.

So it all depends on that particular species how we developed adaptations to a given environment. So the main point is environmental conditions sensed by different organs regulate flowering. So the sensing is done by different organs, like leaves primarily and then root as well as stem all of them are important but the primary sensing is the leaves. And in addition to sensing the environment, some plants need to grow in the vegetative fate for certain period of time. And during that period of time even if the correct environmental conditions are there they are not going to switch into reproductive mode.

So this is called juvenile phase and therefore there is a juvenile vegetative growth to adult vegetative growth. That phase change is required in some plants. This is not common in annuals meaning a plant that lives for less than one year, so there this is not an important point. But some of them like, you know, and a good example you may be familiar with is the coconut trees that grow in many of our house gardens. They do not start producing coconuts right after coming out of the ground within the first year. You know let us say it is going to flower in the fall season.

In the very first fall it is not going to start flowering. So the same environment exists but it does not start producing flowers. So it needs to reach adulthood and that transition it has to undergo before it can actually respond to the environment. So therefore its leaves and roots and other tissues probably lack competence to sense the environment. So we have learned this induction competence concept in animal development earlier that applies here as well.

So this is an important concept we need to remember - juvenile to adult transition. And once that happens then you have another transition, which is vegetative I just told you this instead of producing leaves and stems then it starts to make inflorescence so the apical meristem transitions into what is called floral meristem, sorry, inflorescence meristem. And that vegetative meristem to inflorescence meristem transition is governed by multiple things and in arabidopsis 4 of them have been identified.

One of them is sensing light. This is usually done via a group of molecules called phytochromes and they are important for that. And then there is a genetic pathway that works autonomously so we will learn soon about one of the genes that responds to both of them. And then vernalisation. I told you this is like the stratification we learnt in germination so the plant should have experienced a period of chillness you know the winter and that is required for some of them. So all of them do not work in all of them, some work in some species some do not work in the same species but work in another species.

So vernalization, then a plant hormone gibberellin which is required for germination and the same plant hormone is required to go from vegetative meristem fate to inflorescence meristem fate. Once that transition happens then this inflorescence meristem needs to produce axillary meristems which are actually going to make the flowers. So they are called the floral meristems and there are genes that specify them and they are the hallmark of those cells and they are called the floral meristem identity genes.

So these are more like the homeotic genes, these are homeotic genes but they are not the hox genes we learnt in drosophila they belong to a different group of conserved evolutionarily conserved genes and they function as the homeotic genes here. We will see that in detail as we go along. And once the floral meristem is set apart and these meristems now are capable of activating genes that are going to actually make the different floral parts or the floral organs like petals, sepals, carpels stamen, all those 4 structures.

And these are called the floral organ identity genes. You know, a given gene will be required to make the petals, for example, and its expression would be characteristics of the petal and that is why you use the word identity here, floral organ identity gene. So you have the vegetative meristem becoming inflorescence meristem, the difference here is the inflorescence meristem instead of producing leaves and stems it is going to produce the floral meristems on the axis or axillary meristem.

So that is how the floral meristems form and these floral meristems then activate the production of floral organs by activating the floral organ identity genes.

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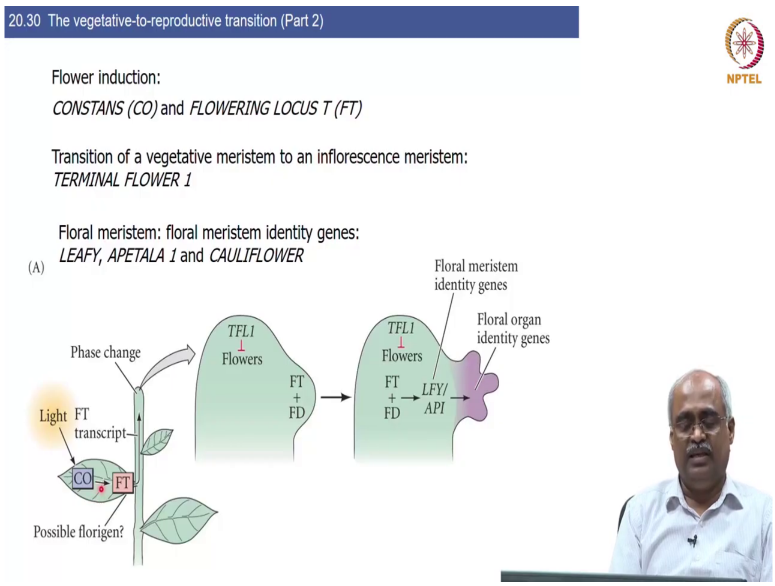

So this is how the flower eventually forms. So here is the genetic pathway that has been understood so far. So the the gene, the products of genes constans is required for sensing light and that in turn activates a gene called flowering locus T or shortly FT and this FT is transported. We do not know exactly how it is transported, mostly the mRNA is transported to the shoot apex that is required for the activation of the transition from the vegetative to inflorescence meristem fate.

And this FT interacts with the transcription factor FD and that induces the formation of the floral meristems on the side, by activating the floral meristem specifying genes the very well understood and important one is the leafy which acts like a master regulator of floral meristem fate. We will see that in a minute, in how the mutant phenotypes look. And they then activate the floral organ identity genes. Another gene that we need to talk about is the transition of the vegetative meristems to inflorescence meristem that is governed by the terminal flower one.

So the terminal flower one inhibits the vegetative meristem converting into inflorescence meristem or producing the flower one single flower at the terminus and that is inhibited by this.

And that has to be overcome by this light induced and other other factors induced, you know ,the four pathways we saw and that needs to be suppressed to transition into the inflorescence meristem fate.

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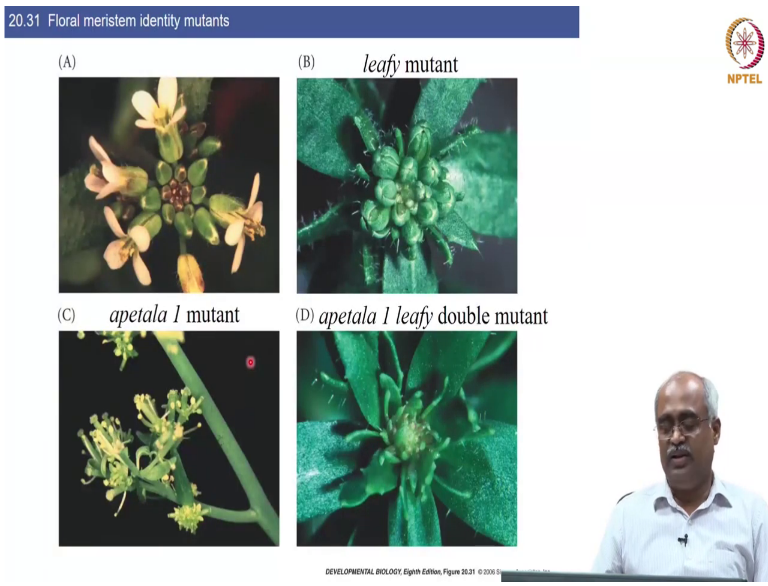

So now how do we know this is how it all happens and you know leafy is really required for floral meristem identity, you know, floral meristem identity how do we know all of that? These are all based on the mutant phenotypes like the name tells you, terminal flower one, meaning if it is not there you are going to produce a flower at the terminus of the shoot. So similarly, if you do not have leafy what happens if you do not have apetala one you know the spelling is here, apetala one or cauliflower what is going to happen?

So these are the things that come from the phenotype of the mutants, so they are shown here. So this is arabidopsis, this wild type flower, you have the nice petals and sepals and you know, stamen is visible there. And this is the leafy mutant, you know it does not produce all the required genes to make proper floral meristem on the axis. And here you have apetala one, in its mutant you have you know, clearly the calyx and corolla are missing so the sepals and petals are not produced. And when you have both of them missing it is like leaf after leaves, so the inflorescence meristem is incapable of producing floral meristems and as a result floral organs do not form.

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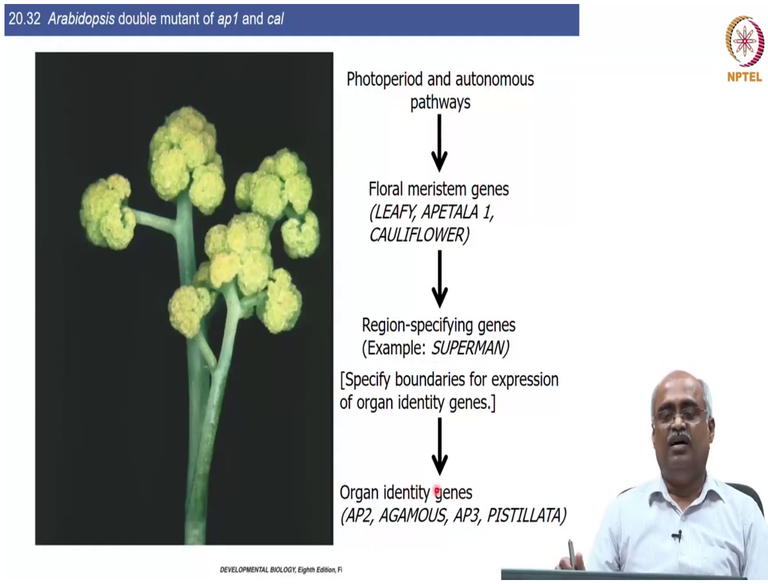

And in the apetala one and cauliflower mutant, so these two seem to function redundantly such that cauliflower single mutants do not have any noticeable abnormality in the phenotype but apetala one and cauliflower together it continuously makes inflorescence meristem after inflorescence meristem and it never produces floral meristem. Instead in that place it makes only inflorescence meristem and that ends up making cauliflower like appearance, and that is why it is called cauliflower.

So the summary here we have is photoperiod and autonomous pathways activating floral meristem genes so this regulation is well understood for leafy gene. So leafy promoter has two distinct regions one response to the photoperiod and another one responds to, you know, gibberellins. And once these floral meristem genes are activated, then they go on to activate a set of genes called region specifying genes, a good example is superman.

And these region specifying genes, what they do actually is they define the boundary of expression of organ identity genes. We will talk about organ identity genes in the next class and where each one of them will be expressed. What is the spatial range in which these organ id genes will be expressed is defined by these region specifying genes, and now these organ identity genes activate the downstream genes required to produce a given floral organ.

So this is how the environmental signals and autonomous genetic signals are all sensed and the vegetative apical meristem is converted into making floral organs. So we will see how these organ identity genes function and what is our current understanding of how they specify the different parts of the flower etc, in the next class.