Plant Developmental Biology
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Lecture - 13
Root Development (Vascular Development)

Welcome back to Plant Developmental Biology course. We are continuing Root Development, in today's class we will discuss vascular tissue development.

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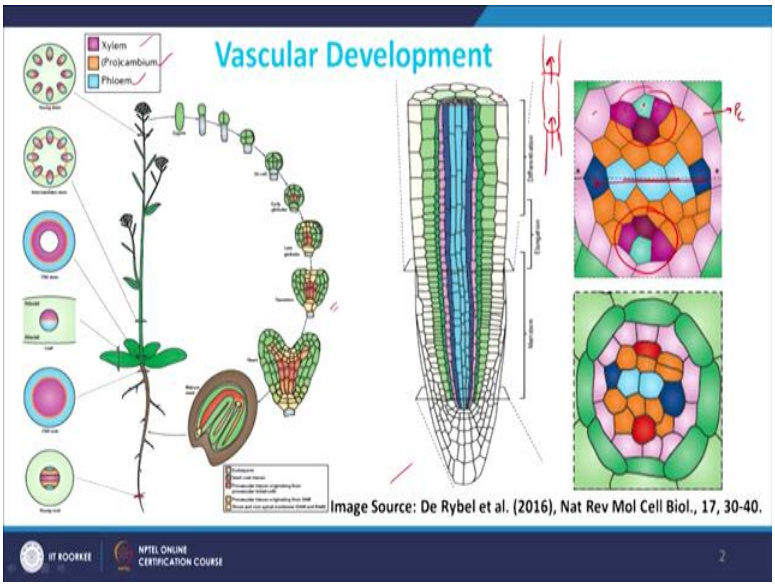

Vascular tissue is important as it makes transport system in the plant. Transport system is absolutely essential for survival of a plant. We also have transport system in every organism, in animals, the nature of transport systems also called circulatory system it is because there is a circulatory mode of transport where we have a central organ called heart.

But in case of plants, transport system is not circulatory it is non-circulatory and it has two parallel tissues xylem and phloem's making the transport system. And xylem takes mostly water, minerals from the soil and it transport everywhere. And phloem takes photosynthetic material and lot of signaling molecules and it distributes or allocate to other part of the plants.

Another important and key difference in the transport system between plants and animals are, in case of animals transport occurs through the tube which is either artery or veins. So, there is a very specialized structure through which the transport occurs outside the cells. But in case of plants the transport occurs through the cells.

So, there are special cells, they have acquired special features and the transport occurs from one cell to another cells and it is moving for both in case of xylem as well and the phloem. And, the vascular tissues have three different components xylem, phloem and cambium or procambiums. Procambiums are stem cells for generating secondary tissues during the process of secondary growth which makes cambium.

The organization of these tissues xylem and phloem is dependent on the organ. So, if you look the root there is a different pattern of vascular tissue arrangement, if you look shoot there is a different pattern of tissue arrangement leaf and everywhere. And the process or the developmental program for vascular development is set at the stage of embryogenesis itself; the primary vascular tissues.

So, as you can see here these tissues are procambium tissues; and this procambium tissues are going to make cambium cells. This is primary root tip and if you look the arrangement of this vascular tissue from the outer side you have epidermis, then you have cortex and then below cortex you have endodermis and after endodermis you have pericycle. Vascular tissue, they have pericycle. In a typical primary root of *Arabidopsis* model plant *Arabidopsis*, below pericycle you have xylem and xylem has a linear axis you can see here and in xylem you have protoxylem and metaxylem, so these two are the protoxylem they are the metaxylem and then at the two poles you have phloem tissues. So, these are the phloem tissues pole and in this phloem tissue, there are two very important phloem tissue these are called sieve element and then companion cells. And if you cut cross section at different places the arrangement or organization of these tissues is different.

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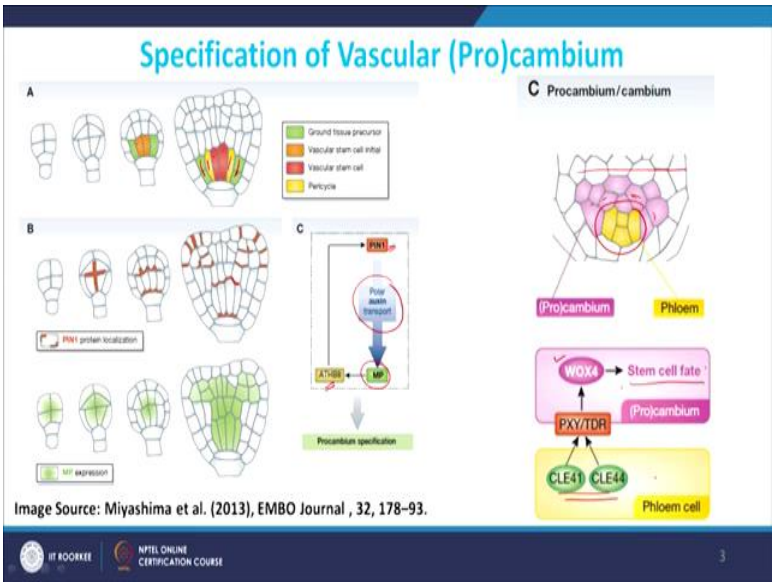

So, the question is that how this vascular tissues are specified, how procambiums or vascular procambiums are specified. If you look the early embryogenesis, so these are your ground tissue initials or the putative ground tissue and then these are the vascular stem cell initials. These are ground tissue precursors; and then you have pericycle and then you have vascular stem cells.

Auxin is very important in all these kind of cell specification and patterning and auxin is polar transported by PIN protein. So, this PIN proteins are very important in distributing auxin helping in a particular tissue or in a particular organ to have auxin maximum. And another important thing for this PIN proteins are that this PIN proteins are asymmetrically localized in the cell.

So, in one cell wall they are localized and their asymmetric localization provides a direction for polar auxin transport because, they are the transport proteins. And if you look at the early stage, auxin is polar transported and this polar transport activates a class of transcription factor this is MP a kind of auxin response factor and this MP activates a key regulator of a procambium identity or specification, which is ATHB8, HB8 is a homeobox class of transcription factor. So, this is how the program of pro cambiums specification takes place at embryogenic stages. In later stages in root how the procambium state or stem cell fate is maintained or regulated? This is a typical cross

section of root. So, this is your xylem axis, this is the phloem pole and these are the procambium tissues. There is a signaling between phloem cell and procambium cells.

So, from phloem cells there are two putative signaling molecule CLE41 and 44 they are being received by the procambium cells through PXY and TDR based reception mechanism. And this eventually activate another class of homeobox protein which is called WOX4 and WOX4 helps in specifying stem cell fate to the procambium.

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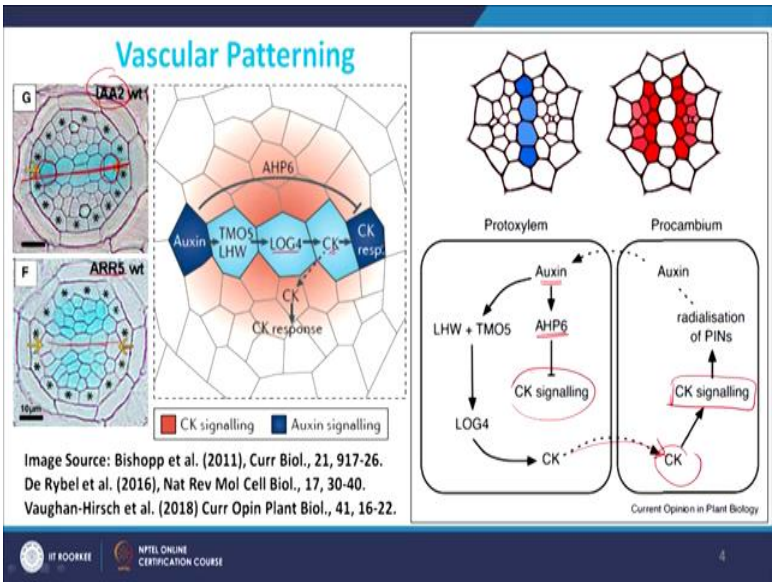

Then how tissue pattering are happening? So, auxin and cytokinin, their antagonistic interaction ensures a proper tissue arrangement in the root. So, if you look how auxin and cytokinins are distributed in the root these are *IAA2* promoter driving *GUS*. *IAA2* gene is target of auxin signaling pathway.

Which means that wherever this expression is there, it means that those cells have activated auxin signaling program. And this is *ARR5* which is response regulators, and response regulators are downstream genes regulated by cytokinin signaling pathway. If you see expression pattern here, you can tell that these are the cells where cytokinin signaling are active. So, if you look here in the primary root, auxin is very high in the xylem axis with little in the procambium cells. And in xylem axis protoxylem are having slightly more expression than the metaxylem.

However, if you see the cytokinin signaling the cytokinin signaling are very less in the xylem tissues, but in procambium tissues the cytokinin expressions are high. And this kind of pattern is antagonistic pattern and defines regulatory mechanism for different tissues. In the protoxylem cells you have a very high amount of auxin and auxin activate AHP6 protein and this AHP6 protein it goes and inhibits cytokinin signaling in the protoxylem.

For protoxylem to be as a protoxylem, there should be very high auxin signaling and no cytokinin signaling or very low cytokinin signaling. Also auxin activates *TMO5* and *LHW* and this activates *LOG4*; *LOG* a gene which play a role in cytokinin biosynthesis and results in cytokinin biosynthesis.

But in the protoxylem, this cytokinin move to the procambium and in procambium this cytokinin signaling is gets activated and this cytokinin signaling in the procambium cells activate all the genes or all the programs which is required for procambium cell maintenance; whereas, this auxin signaling is working in the xylem axis and regulating the differentiation of xylem tissues.

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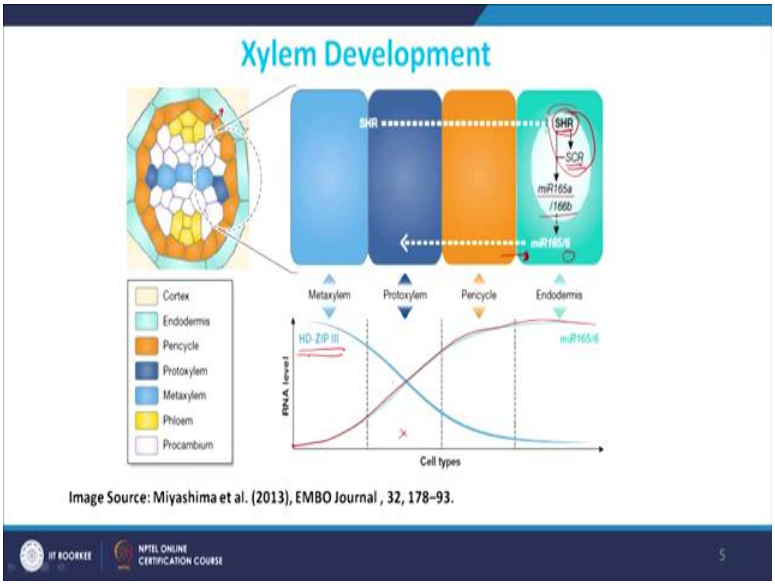

Another regulatory mechanism which works in defining cell fate or identity of xylem cells is through SHORTROOT and SCARECROW protein. If you recall your previous class, SHORTROOT protein is synthesized in the vascular tissues. So, in vascular tissues

you have synthesis of SHORTROOT protein, but SHORTROOT protein moves to the endodermis.

This is the endodermis where SHORTROOT protein moves. Protein is made in the vascular tissues and it is moving to the endodermis. And in endodermis it gets nuclear localized and once the SHORTROOT protein enters inside the nucleus, it activates *SCARECROW*; and SCARECROW and SHORTROOT protein they together activate micro RNA; micro RNA 165, 166. And these micro RNA they are getting produced in endodermis and then they are diffusing back in the vascular tissue and this diffusion, creates a gradient.

The cells which are directly or the cells which are closer to the endodermis will receive high amount of micro RNA and the cells which are away from the endodermis they will receive low amount of micro RNA and that is why you have a gradient of micro RNA; and this gradient of micro RNA is also reflected in the targets of micro RNA. Micro RNA level is very high in endodermis and then micro RNA level is going down once you move through pericycle, protoxylem and in metaxylem.

So, in metaxylem you are expecting very low amount of micro RNA, but in protoxylem you are having high amount of micro RNA. This micro RNA targets homeodomain containing transcription factor PHABULOSA which we have seen in one of the previous class. The metaxylem will have high amount of homeodomain proteins and then the protein levels will go down.

Protoxylem is going to have high amount of micro RNA, but low amount of HD ZIP protein. Metaxylem is going to have low amount of micro RNA and high amount of HD ZIP protein and the amount of HD ZIP protein defines the identity of a cell.

If HD ZIP protein is low in amount, the cell is going to be protoxylem and if HD ZIP protein is high in the cells it is going to be metaxylem. So, these kind of regulatory mechanisms is defining xylem cell identity.

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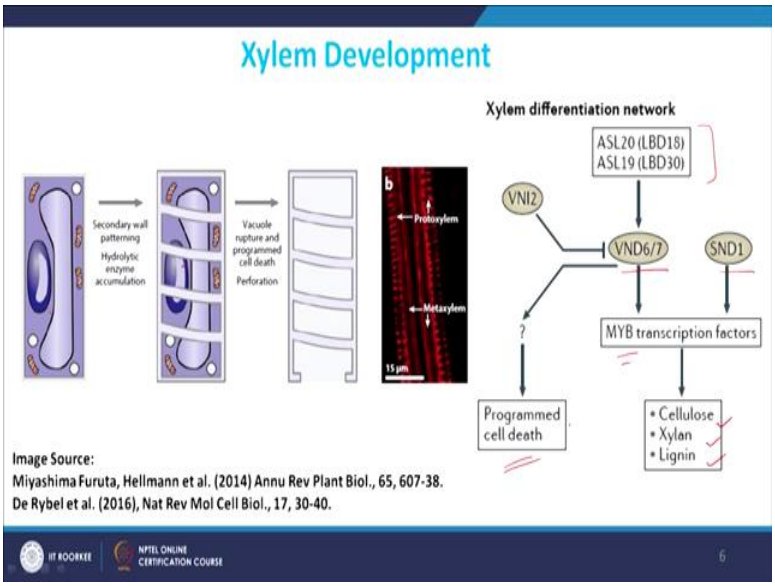

Apart from these specification and identity, there are another very special feature which is associated with the xylem cells which is secondary wall formation. Normally once the cell is specified as a xylem cells, this xylem cells enters in the process of secondary wall patterning and eventually the xylem cells they die through the process of programmed cell death. And this is important, because xylem has to work as a tube for conducting water and other minerals and it has to have a very strong mechanical property.

The process of xylem differentiation is also regulated by another genes. Some of these genes are *VND6* and *7*, *SND1* and these are LOB domain containing transcription factor and they are eventually regulating MYB domain transcription factor. And the interaction of all these proteins ensures that there are cellular modification by depositing cellulose, xylan, lignins. And eventually some of them are regulating the final process of programmed cell death to generate a final functional xylem tissues.

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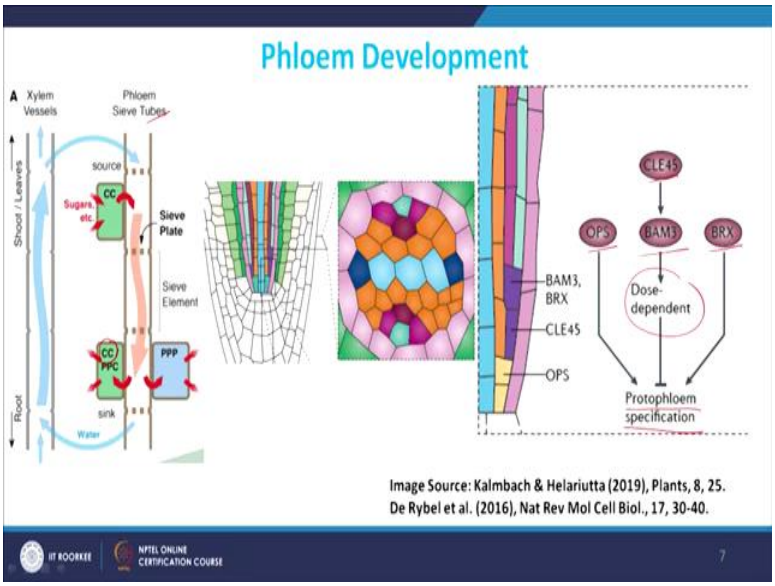

Now, another important tissue which is part of vascular tissues are phloem tissue. This is the picture which tells that they are running in the parallel to xylem. In phloem there are two important tissue one is called phloem sieve tubes or sieve element or sieve cells and this is directly connected with companion cell from one side and phloem pole pericycle from another side.

So, if you make a cross section here you can see that these are the sieve elements, these are the companion cell and these are the pericycle cells, which is directly attached with the phloem pole pericycle cells. And early stage of the phloem development is regulated by the mechanism identified as OPS-CLE40 mediated BAM regulation, working in the dose dependent manner and BRX based mechanism.

So, these mechanisms or these regulators function together to ensure a proper protophloem specification. The phloem at very early stage in the meristmetic zone or upto elongation zones are called protophloems. These protophloem then undergo the process of special differentiation program and this differentiation program is highly regulated again which we will see later. Another important regulator which regulates phloem development is a transcription factor which is called APL transcription factor.

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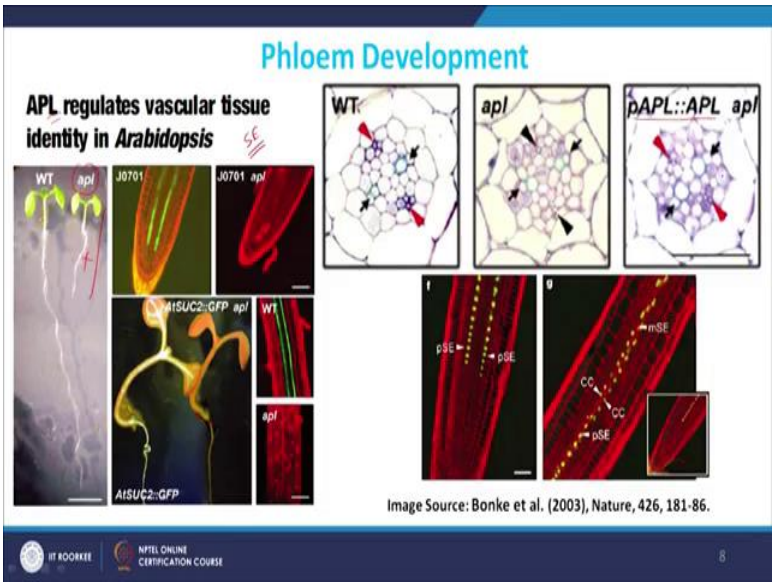

As you can see that regulation of phloem is important if you do not have phloem in this *apl* mutant phloem identity is totally lost and this mutant is seedling lethal. So, plants grow, but it cannot survive for the entire life and if you look what kind of phloems are defective in these mutant background.

So, these are the markers J0701 which marks sieve element or protosieve elements. And if you look in normal wild type root you see this markers are present, but in *apl* mutant this markers are absent which tells that the sieve elements or protosieve elements are missing or there identity is not established.

The SUC2 protein is a marker for companion cell and you can look here that even companion cells are not formed in the *apl* mutants. So, this tells that *APL* regulates both the cell types; sieve element as well as companion cell identity in the root. And if you make a cross section this is your wild type you have xylem axis then you have phloem cells, but if you look the mutant background this identity of phloem cells are totally different.

But if you complement with the *APL* you can see again the normal function. Then you know that *APL* is regulating both companion cell as well as sieve element, but where does *APL* itself express. So, there are two way of regulation; one regulation is that which is called cell autonomous regulation and the other is non cell autonomous regulation.

If the regulator is present in the cell and then regulating its identity; it is called cell autonomous. Protein is present in one cell and regulating identity of the neighboring cell or some different cell then it is called non cell autonomous regulation.

And when you check the expression pattern of APL protein it express both in protosieve elements as well as companion cells. This is nuclear localized signal you can see in the protosieve elements and later stage you can see that this is your protosieve element and the companion cell in the differentiation zone. So, this express both in sieve element as well as companion cells and regulates their identity. So APL is a regulator of both the cells.

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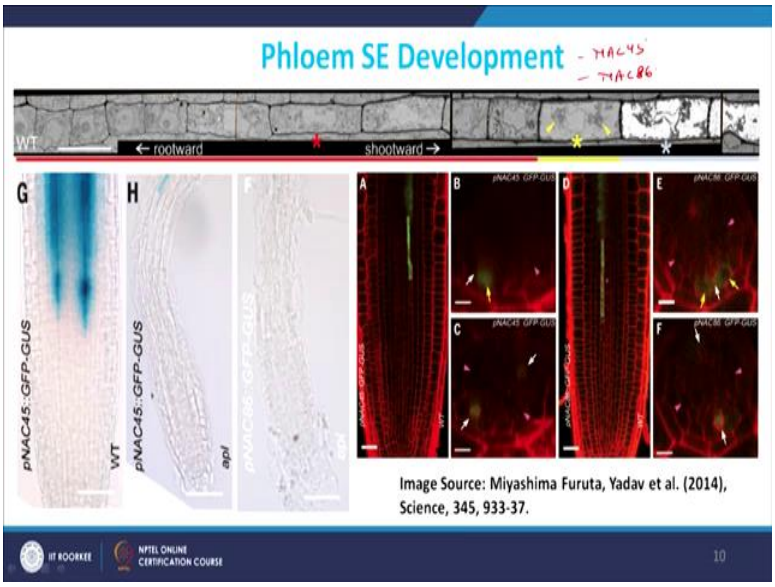

Do we have some specific regulation for the sieve element cell? To identify these microarray was done. But it was performed in a very precise manner. These phloem cells were sorted using a technique called FACS.

FACS is florescent activated cell sorting based techniques. So, if you put some florescent tag in these cells, then specifically you can isolate the cells from these tissues. And, then phloem cells has been isolated from *apl* mutant and then total RNA was extracted and it was used for doing microarray and to identify what are the genes whose expressions are getting affected; and out of these genes two important transcription factors were identified.

So, one was NAC45 and another was NAC86 so both are NAC domain containing transcription factor NAC45 and NAC86. In *apl* mutant *NAC45* expression totally absent. Similarly, if you look here this is *apl* mutant and NAC86 is absent.

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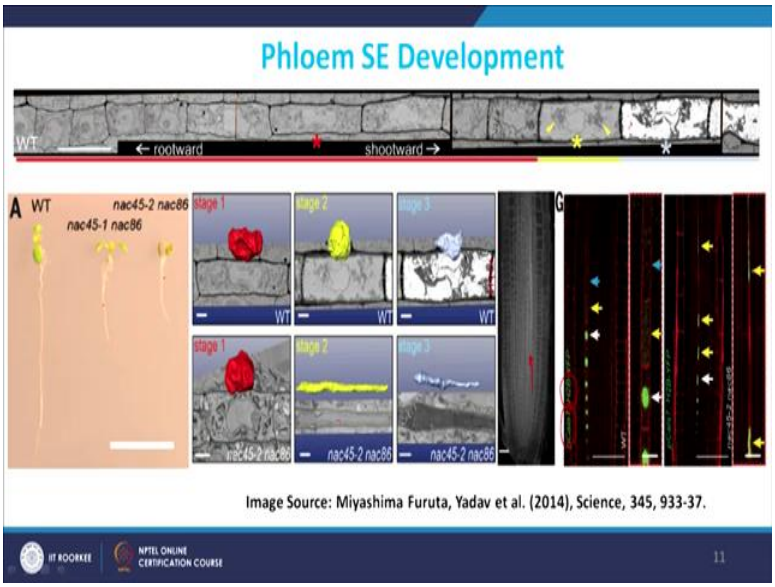

So, this tells that the both *NAC45* and *NAC86* are down regulated in *apl* mutant background. Then if you check the expression of *NAC45* and *NAC86* itself what you find that these genes are very specifically expressed in the sieve element, but they do not express in the companion cell. So, this tells that they might be regulating sieve elements cells not the companion cells and this was true when we make single mutant of *nac45* or *nac86;* not giving any phenotype, but when you make double mutant what you can see that there was a defect.

And then if you analyze this mutants or the wild type pattern of sieve element differentiation in details, if you track one single layer of sieve element you can see this is towards the root tip this is towards the upper side. And at the root tip sides you can see that sieve element cells they are looking very normal in the meristematic zone and then suddenly there is a cell elongation and then some changes started in the phloem cells; and this changes immediately turns in a way that this cells degrades all the cytoplasm including the nucleus.

So, the functional sieve element is enucleated there is no nucleus most of the cytoplasmic components are degraded and this is essential for its function of transport as you know

that transport is occurring inside the cell. So, cell has to have minimum inhibitory components here for the transport system and this happens in a specific manner.

There are three stages; first stage it is very normal, second stage which is called intermediate the process of differentiation or special differentiation of cell sieve element start and then in stage three the differentiation has occurred in a way that the sieve elements has totally lost the nucleus now it is empty like channels and the common cell wall between two cells are very porous in nature, in case of sieve element that is why it is called sieve? Because there are holes there are pores in the cell wall and it makes a structure like sieve and that is important for water or anything to move from one cell to another cell. If you look the mutant this double mutant the cytoplasm and nucleus degradation is blocked.

So, there is no degradation since there is no removal of nucleus there is no removal of cytoplast the sieve elements do not complete its differentiation program and that is why these plants are also seedling lethal they do not survive for life long. This is longitudinal view of your root tip, you can clearly and very specifically see the sieve elements because it has a very special feature, you can see that it has a very thick cell wall which distinguish from other cells. And if you look this is *CAL7* promoter which is specific for the sieve element and if you drive H2B is H2B is HISTONE2 protein.

So, this is nuclear localized protein you can clearly see that till here in the sieve element profile there is nucleus, but here onwards this is the sieve element cells without nucleus. So, nucleus is totally disappeared this is the enlarged view, so if you look here up to here there is a nucleus, but here you can see there is a diffused GFP signal which means nucleus is under the process of degradation. And then just next cell here you can clearly see that nucleus is almost removed.

But if you look the mutant in mutant the nucleus is not degraded even if you go to the very higher side of the cells where cell, sieve element cells are very very long, but you can still see the nucleus.

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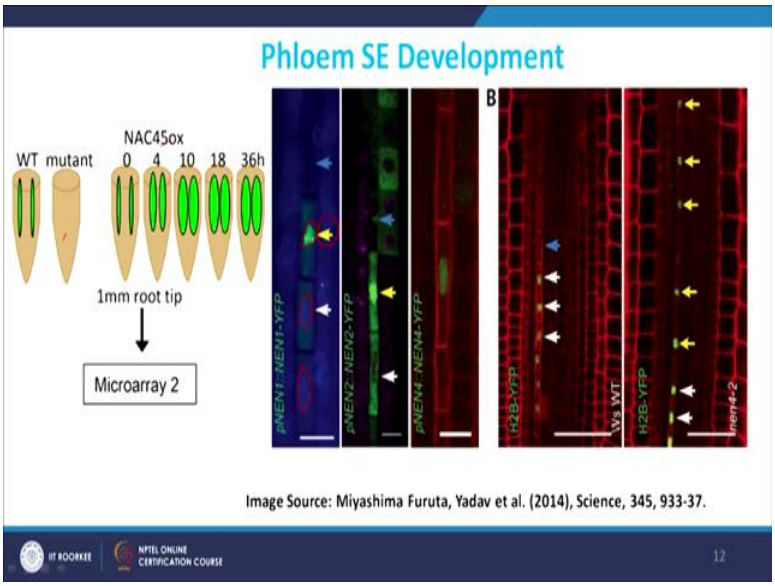

This suggest that in the double mutants nucleus is not degraded. Mechanism regulating the process of nucleus degradation was further identified. *NAC45* and *NAC86* is regulating nucleus degradation, but it is a transcription factor, it cannot go and directly regulate the process of nucleus degradation, it must be regulating some of the genes which is directly involved in the process of nuclear degradation. To identify these genes another round of microarray was performed using this double mutant and *NAC45* overexpression.

And from this microarray analysis four genes from a small family of putative nuclease family was identified which was *NEN1*, *NEN2*, *NEN4*, *3* as shown here. These genes are expressed in the sieve element which is correlating with the fact. In early sieve element or young sieve elements the gene is expressed in the sieve element and protein is made, but protein is not able to enter inside the nucleus this is the nucleus and protein is entering only in the cell where nucleus has to be degraded.

Before this protein is not able to enter inside the nucleus so there are two regulation one regulation is that this nucleus genes are getting expressed in the sieve element that is regulated at the transcription level and then another regulation is at the post translational level when protein is already made its translocation to the nucleus is regulated. Similar kind of things you can see for *NEN2* as well where as if you will see *NEN4* is specifically expressed only in the cell where nucleus has to be degraded.

So, if you look early cells does not express later cell nucleus is degraded. So, there is no nucleus no signal, but this cells has NEN4 protein localization only inside the nucleus. So, all this story tells that this putative nucleases might be responsible for nucleus degradation. And this is supported genetically when you have *nen4* mutant background you can see that nucleus degradation is defective in this mutant background. So, here everything is normal except *NEN4* is not present.

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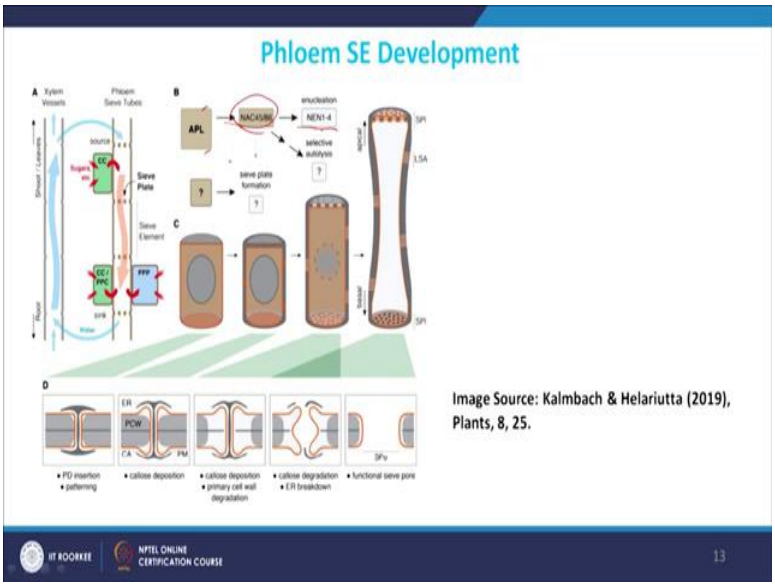

This all suggest that *APL* is regulating both sieve element as well as companion cell. And during this process *APL* is regulating *NEN*, *NAC45* and *86*. *NAC45* is regulating sieve element differentiation cytoplasm as well as nuclear degradation, by regulating *NEN1* to *4* and this NEN is specifically regulating nucleus degradation. This is how the process of phloem specification takes place to make a mature phloem, mature sieve element cells. This mature sieve element cells is very special and has sieve pore like structure which is almost empty, so it is very suitable for very high or efficient transport system.

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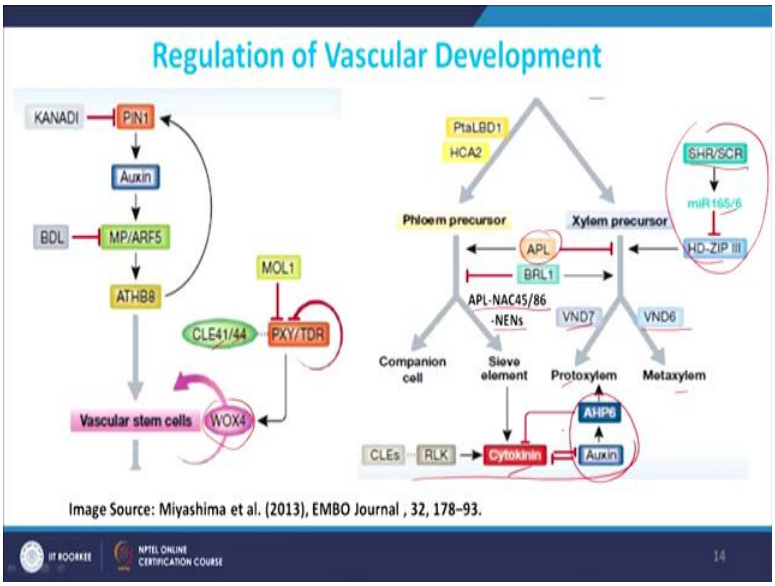

To summarize the vascular development, so what we have studied. Auxin is regulating auxin response factor MP which is regulating ATHB4 and this process is ensuring vascular stem cells through the *WOX4* genes. There is another regulation from the phloem sides which is CLE1, CLE41 and CLE44 mediated regulation and it is working through a PXY/ TDR to regulate this stem cells identity.

And then later on there is a mechanism through SHORTROOT protein and SCARECROW protein, micro RNA and HD ZIP mediated protein. This is regulating xylem cell identity, protoxylem versus metaxylem identity. Another regulator is *VND7* which is regulating protoxylem identity *VND6* regulating metaxylem identity.

But on the other hand *APL* is activating phloem identity, *APL* is regulating companion cell and sieve element both and for sieve element based specification it is using *NAC 45*, *NAC 86* and *NEN* mediated signaling pathway. And then protoxylem is also regulated by the feedback of auxin, *AHP6* and cytokinin based mechanism.

So, will stop here in class we will discuss root branching.

Thank you very much.