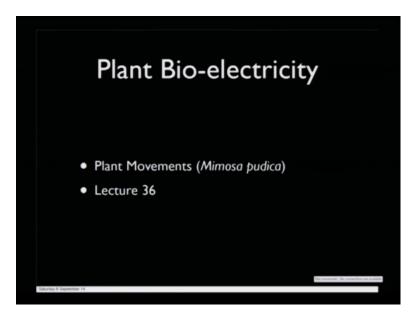
Bioelectricity Prof. Mainak Das Department of Biological Sciences and Bioengineering Indian Institute of Technology, Kanpur

Lecture – 36

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So, good evening everybody, so today we will be starting the thirty-sixth lecture followed by the thirty seventh lecture. So, in this section, we will be talking mostly about the plant bio-electricity. So, we have talked about photosynthesis in depth, and we have talked about how the electron transport is taking place from photo system 1, photo system 2, and how the water splitting cluster or the manganese cluster is working. And how people are getting or deriving inspiration from all those things to derive different kind of molecules which will be helpful in obtaining energy.

So today will be talking about some of the most fundamental plant movement which are bio-electromechanical movements. So, in this section, we will be taking up two examples; in this lecture, we will be talking about touch me not plant which all of you must have seen at some point or other, where if you touch the plant, it bends. So, after sometime it regains its original position, how that happens. So, if you just from knives perspective if you look at this situation, it is something like as if, when you are touching the plant you are creating a pressure on the surface of the plant, (()) is not every plant is adapted to it. There are very few plants which are adapted to it, and we will be only

talking about one classic example on which extensive research has taken placed in last hundred years or so.

So we scientifically called Mimosa Pudica or in common name it is called touch me not. So, from a very knife perspective as I was telling you think of it, it apparently seems that these kind of plants have a pressure sensor. So, whenever you are touching the plant, it senses a pressure, and based on that pressure sensor, it does certain action - some mechanical actions. And this is fairly similar to something what happens? When you are hearing. It is a sound wave coming, hitting in your ear drums, followed by the wave enter inside the cochlear, and it opens up a series of ion channels in the hear cells of the cochlear ,and thereby leading to the electrical activities, which is coded by the brain, and you realize what you are hearing. It is pretty much the same thing. And these studies lead the foundation stone for you know in a controversial topic called the nervous system of a plants are these.

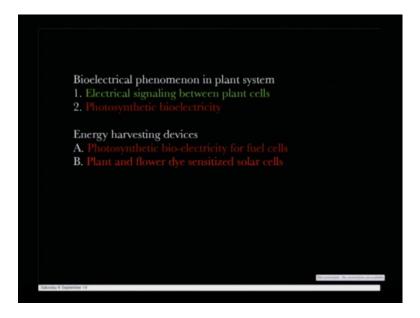
So, these are open ended questions, are these kind of sensing abilities something there are very similar to you know stretch reflex arc what you have already studied in the nervous system or the hear cells in the ear. Or these rudimentary activities are kind of you know very under developed nervous mechanism these are, because here is no structured nervous system in the plant. What we know or what we have already discussed while introducing the plant that it is a structure of tubes of xylems, and the vessel, xylem vessels, and phloem vessels, one of them carrying the water, and others solutes. Whereas the other one is carrying other bigger molecules, and supplying nutrients all over the plants, and clearing of the debris, and everything.

As such in anatomy of the plant there is nothing as such what you see in the animal counterpart as nervous system yet they behave very similar to the nervous system, and if we really go through this this lecture you will realize that this is inspired by generations of scientist in order to understand these kind of sensors could you emulate those sensors with the modern techniques of nano technology, and bio-electromechanical systems bio mems, and all these things could you really need those kind of pressures sensors. And what are the sensory structures apart from it from the very fundamental perspective these are of enormous significance how a simple touch sensation in a plant leads to this mechanical action overall what happens is what will be discussing today these touch sensation or the pressure sensing leads to a cascade of electrical responses those

electrical responses eventually translated into a mechanical response. So, this is the nature bio-electromechanical system.

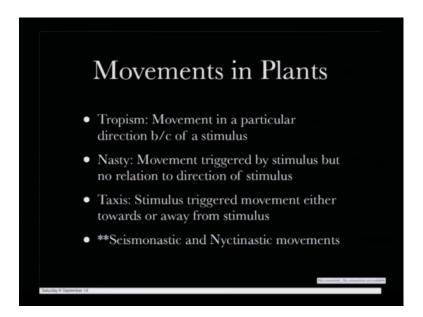
So, let us formally start the class. So, then this section of plant bioelectricity will be talking about the plant movement or the mimosa pudica, and this is the lecture number thirty six.

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So, now, let us see what are just rehash of what we have already done in the bioelectrical phenomena plant systems today will be talking about what you see in green the electrical signaling between plant cells, and other three topics two we have already done, and will we have already also done the the dye sensitized solar cells.

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So, let us progress with the electrical signaling between the plant cells. So, there are different kind of movements which are observe in the plants. So, one is called tropism tropism is a movement when the plant moved towards a basic stimulus say for example, phototropism photo means light. So, if the plant kind of directionally move towards sun light we call it phototropism you may see you know the sun flower are pointing towards the sun light whichever direction you put them suppose they are in a you know in a in a not in the ground.

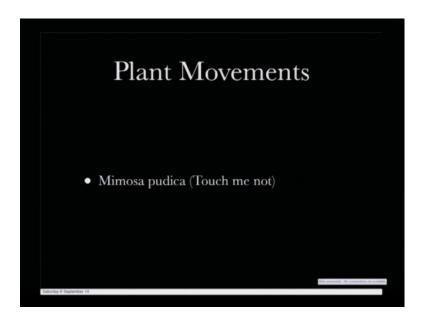
I mean you know some kind of a, and you just tilde the direction, and they will try to move towards light they will try to you know obtain as solar energy as possible this is called tropism heliotropism phototropism likewise gravitropism towards the gravity, and likewise, then there is something called nasty nasty is basically the movement triggered by stimulus, but no relation to the direction of the stimulus. So, this is. So, this basic difference between tropism, and nasty is in the case of nasty you do not have any directional or vector sense.

So, if there is a sensation of there is a stimulus they will respond to the stimulus where as in the case of tropism the directionality component matters depending on the position of the sun the plant is orienting itself in order to you know derive as much solar energy as possible it is just one of the examples the third one is the taxis the stimulus triggered movement either towards or away from stimulus. So, if you look the compare between tropism, and taxis when you talk about tropism you say it is direction, because of the stimulus it is moving towards the stimulus.

But in the case of taxis it is basically can move away from the stimulus say for example, there is an obnoxial stimulus something which a plant does not like it will move away from the stimulus. So, that is called the taxis, and the last one which we are going to deal with is called seismonastic, and nyctinastic movements I am not getting into that at this point, because that is what we are discussed away. So, broadly speaking these are the different kind of movement if you really correlated really simple in a very simple sense.

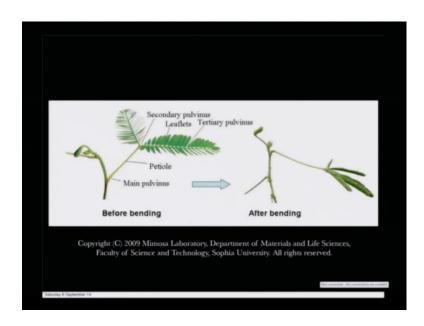
We look towards the light our eyes respond to it as if the plant responds to the sun light as if they have you know photoreceptors, which are sensing towards them, and that is all coordinated motion you can always imagine that like you know there are certain rudimentary electrical impulses getting generated rudimentary nervous system which is functional out there. So, based on these four movements towards or away from the stimulus or irrespective of the directionality stimulus, we are will be moving will be talking about this seismonastic, and nyctinastic movements.

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So, this is our example what we are going to deal with mimosa pudica or touch me not plant.

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So, here I have collected some simple pictures those of you have seen mimosa pudica plant is fine, because during the rainy season you will see lot of bend in the woods, and in your backyard they grow pretty much all over the place if you haven't seen they are very similar to the picture what you see, and if you have the nack really go to the garden, and kind of see trace these plants you can trace them you touch it, and figure out whether it is a touch me not or just another ordinary plant.

So, this is how it looks like, and if you look at the structure very carefully the... So, here it has bend shown how it is bending. So, the main frame is like the from the main stem you see the main pulvinus, then the petiole, and this we have already discussed a, then you see the secondary pulvinus you see the main pulvinus secondary pulvinus, and there is something called a tertiary pulvinus. So, there are three and. So, there are reason why I am stressing on this pulvinus is this these are the regions where the bending is taking place ok.

So, is the pulvinus region where all the it is just like the if you look at my hand. So, this is the zone where the bending is taking place or this is the zone where the bending is taking place. So, similarly for the leaf. So, the first is the tertiary which is the tip of the leaf the leaf will curve, then the next one is the secondary pulvinus where second level of bending, and the third one is the main pulvinus, and if you see after bending. So, if you look at these two compare these two photograph, you will see the all the three bendings

have taken place in the main pulvinus in the secondary pulvinus as well as in the tertiary pulvinus ok. So, this is how all the bending actions takes place, fine.

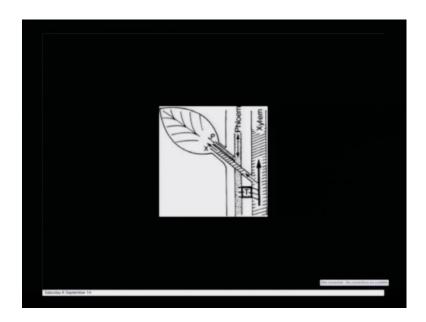
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So, now, let us take a very closer look at it, because in that picture what you saw just before this it is not a its kind of a distance if you have a slightly closer look you will see the bending is taking place at the if you see that is in the red it is mentioned pulvini. So, it is at the pulvini where the leaves are. So, it is the leaves remain like this as soon as there is if you touch this plant it curves like this. So, there is there is needs a lot of like you know flexibility in this motion it is not every leaf does it.

So, having seen this. So, at least one thing is clear your pressure sensors are sitting somewhere along these leave surfaces, and these leave surfaces are receiving it is a nutrients, and everything through the xylem, and the phloem.

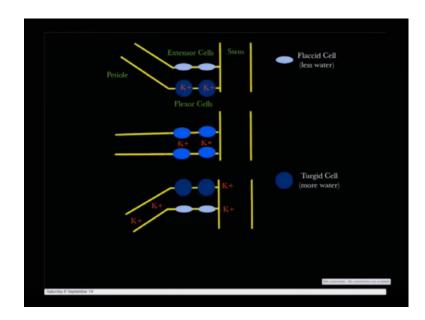
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Now let us move on to the just small recap of about the xylem, and the phloem vessels. So, you see as I told you that basically these are cylindrical structures along the stem of the plant one is specialized for carrying all the water molecules which is the xylem vessels, and the phloem vessels which is a specialized to carry several other nutrients, and everything ok.

So, this is how the leaf structure is supplied with xylem, and phloem also, and you see there is a tea connectivity. So, there are zones where the xylem, and phloem vessels has limited connectivity across those cylindrical motions now after this small recap you guys remember this I o one of lecture I told you that I am going to in order to describe the whole structure.

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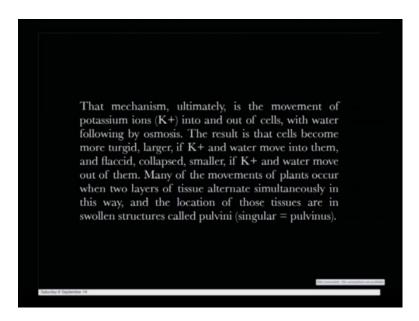
So, that you know there is no confusion, because this is very essential now from here now let us see the situation now what you see here in yellow is kind of I have drawn this structure in a very simplistic way. So, yellow what you see is the stem.

So, if you want to correlate it. So, you can coordinate it with this structure. So, this what you see here is the petiole or the or the secondary pulvinus. So, imagine all that yellow thing what you seen here is a secondary pulvinus, and the petiole there. So, first set is the situation when there is no water in the cells it is called flaccid cell less water. So, now, in the first picture there are three pictures here you touch the cells once you touch the cells your end result what you see is the third one. So, what essentially happens it has been figure out when you touch this cells it opens up for series of potassium channels which are present in the plant, and from the extra cellular space potassium started to enter inside the cells.

And when the potassium enters inside the cell this along with it carries a lot of water molecules, and once it started carrying the water molecule what happens now I will just take you back before I come to this one this picture. So, imagine this is before bending you touch once you touch lot of water molecules gets accumulated inside the leaf, and, because of the weight of those water molecules the leaf from this position droops down like this. Now let us coming back to the mechanism, and one more thing why this picture is essential.

Because, then you will be able to correlate how the water movement is taking place you see the x, and p is x standing for the xylem, and the p stand for the phloem vessel now coming back. So, this is the situation one when your cells are without any water. So, flaccid cells. So, now, when you touch all those you see those k plus k plus channels which are sitting there they open up, and they allow a lot of potassium to enter inside the cell, and along with potassium what happens you're allowing a lot of water to enter inside it, and that makes the cell extremely turgid, and once the cell becomes turgid at you know this droops down like this ok. So, this is the overall understanding of the mechanism what happens in the case of touch me not plant.

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So, now, just to you know put it together the mechanism ultimately is the movement of potassium ion into, and out of the cells. So, this is the regulated mechanism with water following by osmosis the result is that cells become more turgid larger if potassium, and water move into them, and flaccid or collapsed smaller if potassium, and water move out of them many of the movement of plants occur when two layers of tissue alternate simultaneously in this way.

And the location of those tissues are in swollen structure called pulvini. So, that is the reason why I was telling is you have to take a very clear look at this picture. So, this is the pulvini or pulvinus why these kinds of tissues remain. So, one allows twenty the other one allows the reversing, but now what is important you have to know if this kind

of motion takes place or movement take place of these ions you can actually record these electrical motion this is nothing, but just only the difference here is from the animal electricity is that most of or all over animal electricity what?

Whatever we know leads to a influxed of sodium ions here in the case of plants you see potassium plays a very significant role in terms of regulating. So, if you could poke an electrode in between. So, you say for example, if you could place an electrode somewhere out here between the like between the extensor, and the flexor cell. So, there are two kinds of cell you have to realize here there is something called the extensors cell, and there is something called flexor cells if you see this picture very carefully you see both the cells are given there. So, those that is what is meant by two kinds of tissues many of the movement plant occur in two layers of tissue alternate simultaneously.

So, now if you put in the electrode out here you should be able to record the electrical current which is being generated by the ionic flux of the potassium or any other ions which are involved in it.

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So, now, coming back now will have will go back to some of the historical perspective when this all started. So, this is a paper I wish all of you can download it online, and this was a paper which is published in nineteen sixty two twenty nine January in the science journal, and it was published in Japan excitable cells in mimosa, but if you see the references cross references.

You will see the third which is very important to look at by j c bose back in nineteen twenty six when he propose this idea, and he made some very, very seminal contribution. So, what I was trying to tell you for last almost more than hundred years this research is going on, and I will tell you how bose meet some of those studies which eventually followed by takao sibaoka on which the people what has been sited out here.

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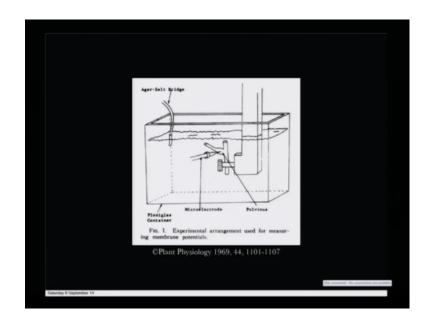


So, this is what takao sibaoka was look for it please go through this paper; however, the experiment by bose in which an electric probe. So, look read through these lines very very carefully. And that is why I am kind of repeating these lines with you people in which an electric probe was insert inserted into the petiole at various depths. So, what that essentially means is if I take you back to this picture. So, you're probing at different depth you know at different depth to see where you are getting the electrical signals you can just like say for example, you take you take suppose this is your electric probe. So, you go up to this depth you go up to this depth you can go up to even further depth based on the depth you are measuring the electrical electrical fluxes. So, this is what exactly bose was dream at various depth showed that excitation is conducted not only in the phloem.

But also in the protoxylem located in the inner part of those xylem the protoxylem was called the internal phloem by bose, but this tissue consist only of elongated parenchyma cells, and contains no sieve tubes now sieve exciting cells in my experimental I inserted

microelectrode into the intact cell, and found excitable cells in both phloem, and protoxylem. So, essentially this was a quantum jump nineteen twenty six with bose published is work on or documented is where in a book called nervous system the plant in nineteen sixty two there was almost forty years later.

So, this story is still continuing there are people who passionately follow this thing you know plant indeed produces electricity, and we can really measure those electricity's using in the micro electrode, and if you see the experimental set up.

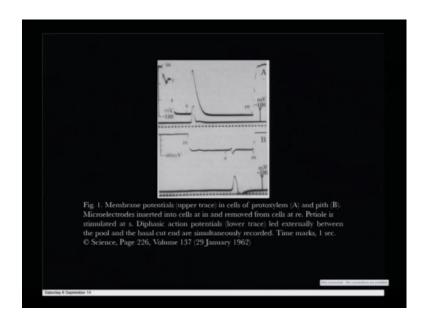


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It is something like this. So, if you see the pulvinus how the... So, basically this whole thing is submerge in a in a electrolyte where the conducting. So, now, you you fix it using a if you see this picture you see the pulvinus is fixed in a kind of you know what is something like a cramping you cramped it, and from the left hand side you see the micro electrode is being inserted pending of the depth of the micro electrode current density changes.

And this is how this whole experiment was conducted by sibaoka, and much before that by bose, and all these people these are very simple set ups, but these seminal studies made the foundation stone for a totally different world of electrical responses of the plant, and if you see some of those recordings.

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These are some of those recordings from some sibaoka's paper. So, I have already exposed you people to the action potential you seen in the case of animal cells, you know here is an example of action potentials which are observed by the plant cells, and depending on where the electrode is your action potential changes.

And if you see the resting membrane potential you see the resting membrane potential is sitting at minus one eighty milli volt unlike your plant animal counterpart where it set minus ninety or minus seventy or minus eighty milli volt or in the case of pacemaker cells or few of the you know yeah some of these pacemaker cells. So, sitting at minus forty. So, with respect to the animal cells if you compare these value this is way in way more negative this is sitting at minus eighty, and this spikes what you see in this pictures are the spikes which is which are telling that you know there are electrical activities based on the touch.

So, this is what if I have to tell you. So, this is how it is being done. So, say for example, I insert an electrode here though this is the plant this is the leaf, and I insert an electrode like this fine I touch I did do a recording here depending on what is the depth I have I am getting different kind of recording now if you refer to the slide, and that is exactly what you see if you refer here the membrane potentials in the upper trace in cells of protoxylem. So, that is that will was a contention of sibaoka, and the pith which is in b please follow the b where you see microelectrode inserting into the cells at in.

And removed from cells at re... So, that re, and a stands for that when you are again inserting petiole is stimulated at s you could see the s very clearly when you give the stimulation, and diphasic action potential lower trace led external externally between the pool, and the basal cut are simultaneously recorded. So, you can if you see this picture, and if you go through this original paper in science everything will be clear. So, just for your understand before you read through the paper this is how it works.

So, this is say for example this is the leaf coming out from petiole insert the electrode like this, and depending on the depth you are giving a touch with something some other thing you know some other thing which is clamped here giving a touch you making a recording giving a touch you will making a recording.

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+ 12	-19 = 13
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	2 = 8 = 15 = 1

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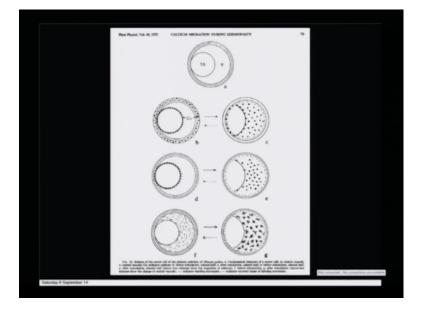
This is how all these recordings have been established, and based on the first recording it thing without the touch it resting on your potential. So, this is something which is if you now compare the resting membrane potentials. So, this is something really interesting to look at say different tissues of the plants have different resting membrane potential.

Say epidermis sitting at minus forty four which is very close to the peacemaker cells cortex minus fifty two again close to the peacemaker cells of the heart sclerenchyma sheat minus fifty two phloem small cells are sitting at minus one sixty one, and minus sixty one see the variation in the plant protoxylem is sitting at minus one fifty four pith at minus fifty eight reason why I am putting all these things is, and if you see the shift from

when there there is an action potential you see that asterix, which is shown that basically telling you from one sixty one it shifts all the way to twenty two.

So, you realizing that there is almost eight times reduction in the in the membrane potential similarly in the case from one fifty four it goes to minus nineteen. So, just like their animal counterpart these kind of action putting cells are been observed in the plants, and this is a, these are those seminal studies from the time of bose all the way seabioka, and further down which is later foundation stone to believe what bose claimed is long long back that plants indeed happened very rudimentary nervous system, and with more, and more studies with more, and more technologies will be able to unrawle those extraordinary field whch has been achieved by plant ok.

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So, after this what I will do? So, this is another thing this is again from the same paper in I am sorry another paper in nineteen seventy two yes can go through it how's that here you could see how that dripping is taking place you know the drip, and how some of the vacuolated structures inside the inside the xylem, and the vessels are changing, and this is one of the model you can go through this model there is the calcium the role of the calcium, and I giving you the reference also in the pan physiology, but at this stage I expect that. So, essentially what its telling is the calcium flux along with the potassium.

Which is also involved in it if you read through this fundamental structure of the motor cells you will realize that just like your motor neuron we have this motor cells, and

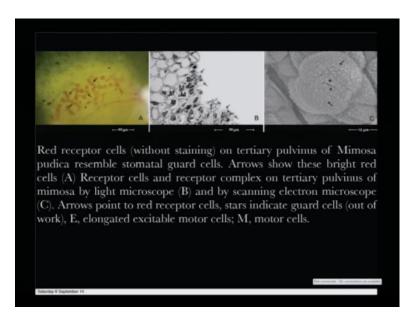
everything which are doing orchestrating this whole process. So, there is now we are adding one more dimension to it along with potassium there are calciums which are involved in it. So, this is what is the situation before stimulation, and this is a situation after the stimulation as a potassium comes. So, integrated scheme of the function of the motor cell at effexial half of mimosa indicates bending moment indicates recovery phase after the bending moment.

And this is the kind of broad view, and this is the kind of a small view, and I kindly request you people kindly go through this nineteen seventy two paper by toriyama, and jaffe in the volume forty nine now next we are coming on to. So, we wade these recordings now this brings us back to a very philosophical situation if you remember I told you that when we talk about the pressure sensor like we talked about there is a pressure sensor which senses it a sensor in neuron which carries the message, and which is being processed in the spinal cord, and brought back, and tell the muscle to come back to its original pressure compression.

So, now in this whole thing what e just covered in last twenty minutes all we haven't talked about any pressure sensors where is the really the pressure lies there has to be a sensor something which is sensing this whole thing, and who is the sensor we have talked about the extensors, and all those cells, and everything exactly how the sensor looks like. So, now, we will be talking about the mechanoreceptors the the the one which acts or which reacts upon touching the leaf. So, if you look at cells look at the cross section of a leaf what will you observe is there are certain very specific cell called red receptor cells.

So, on the surface of the plant you know if you take a section you will see there are very specific structure called guard cells which regulates the moment of the water inside the leaf, and outside the leaf, and the most of the time in a hot sunny day the guard cells remain closed very tightly regulated, but on a kind of you know very rainy day they remain open you know they wanted to get redo excess water.

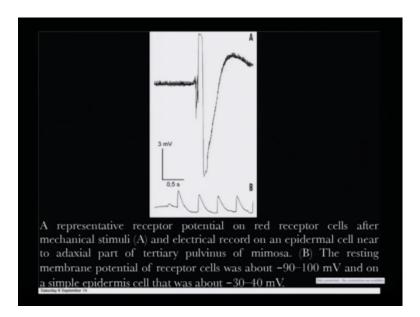
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So, very similar to the structure of the guard cells as it you know something like this structure like this there are another set of structures which are called red receptor cells that you could see upon standing on the panel.

A you could see receptor cell without any, and this this is without standing by the way can this is seen in the tertiary pulvinus of the mimosa pudica resembles the stomatal guard cells arrow shows these bright red cells receptor cells, and receptor complex on the tertiary pulvinus or mimosa by light you can just use a simple light microscope, and by scanning electro-microscope arrows points to the red receptor cells stars indicate the guard cells which are out of work, and if you look at them an elongated excitable motor cells. So, we are talking about these motor cells just in the previous slide we were talking about, and the calcium motion, and everything. So, this is how this whole thing looks like now representative receptional potential of the red receptor cells. So, essentially what happens is that if you really could do a recording from these red receptor cells what I have been shown here you will see there is a sharp upon mechanical stimulation and. So, the way you are doing it you are doing a recording. So, you have your electrode like on those red cells you are placing an electrode on those red cells on those red cells you have the electrodes sitting on those red cells. So, you have electrodes sitting on the red cells.

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And you are touching the plant on touching what you see this is what you see upon touching a representative receptor potential on red receptor cells after mechanical stimuli the electrical record on an epidermal cells near an adaxial part of tirtiary pulvinus of mimosa resting membrane potential of receptor cell was about minus ninety to minus hundred milli volt, and on a simple epidermis cell that is about minus thirty to forty milli volt. So, there is always a shift on that. So, these are some of the some of the location of the real pressure sensors who's counterpart issues see in the animal kingdom their counterpart is the muscle spindle ok.

So, this is this slide I am just putting here for our you know better understanding about the detailed structure how these the xylem lamella flow in the cells, and how they are integrated into the structure the plant cell wall, and everything plasmadesmanta just this is a kind of a recap, and this is where we are talking about the receptor complex on tertiary pulvinus by transmission electron microscope r what is stands for the red red receptor cells, and g stands for the guard cells, and e p are the normal epidermal cell ok.

And any of the excitable motor cells. So, this is how this whole complex structure looks like. So, r for the red cells plasmodesmanta arrow is been shown you see there is a there is an arrow on the panel b where there is a connectivity, and [elong/elongated] elongated excitable cell are shown in shown out here you could see those e's sitting out there. So, now, this is the complex which is responsible for. So, whenever there is a touch on those red cells which are acting as a mechanoreceptors they convey this message to these motor cells, and that leads to the influx of a series of potassium ions as well as calciums in, and around it.

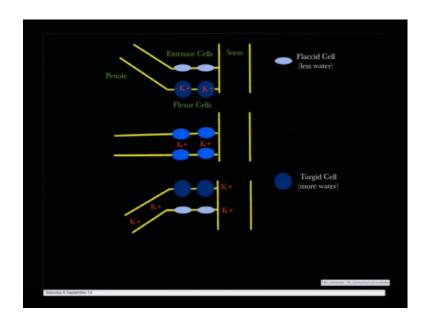
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And which leads to this process of drooping of the leaf. So, it is a very interesting event, and slowly slowly we are kind of you know getting an understanding of what is happening. So, here for your those who are keying to really you know go through it I will put a model for you guys you can go through those office papers which I have already sited you can go through them how this is this whole thing is taking place. So, again to just summarize what we just talked about just from the. So, this is where we started, and we were suppose to talk about the sysmonastic moment.

So, this is all the pulvinus looks like. So, on those pulvinus we talked about how to place electrodes at different level, and there are two specific kind of a structures one which is called the sensor, and we talked about the senor structures out here once again these are the sensors red receptor cells, and you have a series of cells here which we call them as extensor, and flexor cells kind of the motor neurons their counterparts in the animals animal kingdom.

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So, whenever these red receptor cells are activated they through the plasmadesmanto connect to these motor cells or extensor or the flexures. And lead to the moment of these ions inside that, and if you clever enough to put the electrode in the right place, then you will see you will be able to make the electrical recordings from this from this tissues. So, this is the overall geometry by which a mimosa pudica bending is talking place that is why I told in the begging that this is an inspiration for a series of bio electro mechanical systems where if you see this picture. So, all you want the most elegant cells are setting there you touch the sensor sensor is all over place you touch it, and that leads to the bending.

So, if you really could have those these kind of system you could emulate using the modern tools of nano technology, and you know micro fabrications, and everything really can do some really some wonder wonderful sensors which can develop over the years. So, I will close this this lecture out here, and our next lecture will be on the another kind of plant moment which is in the veinuslidra.

Thank you.