Speaker 1 (00:15):

Hello everyone? And welcome to the Illinois X Space Technology Talks presented by the Illinois Space Grant Consortium, The University of Illinois Urbana-Champaign and Northern Illinois University. Today we have Darron Luesse and Dr. West Swingley. Our first speaker is Dr. Darron Luesse. He is a molecular plant biologist. He received his bachelor's from the University of Missouri and his PhD in the plant biology from Indiana University. Dr. Luesse completed his post doctorate work at Ohio university, and he has been a faculty member in the department of biological sciences at Southern Illinois University in Edwardsville for the past 12 years. Dr. Luesse has a variety of research interests, but mainly focuses on plant phenotypic plasticity. Has a strong interest in how plants as [inaudible 00:01:10] organisms can sense the world around them, then change their growth patterns to maximize any available resources.

Dr. Luesse has mostly used gravitropism to model this. In 2015, he was even part of a collaboration to send seedlings to the space station aboard the BRIC-20 mission. These seeds were then used to get a transcript modic, I'm pretty sure that's how you say it. And [inaudible 00:01:40] profile of dark grown seedlings and microgravity. Today Dr. Luesse will be talking about how plants change their growth and development in space, as well as how to study the molecular changes that underlie these processes. You'll probably be able to say the words that I was trying to say a lot better than me as well.

Dr. Darron Luesse (01:58):

All right. Thank you for the introduction. Yeah, hopefully I get all those words right. Today I'm going to talk about how plants grow and develop in space. This is part of my interest of what I like to think about on earth and in space plants have new challenges. I'm going to talk a little bit about what those challenges are and then some of the techniques that we can use to understand how plants grow and develop. The first thing, if you take nothing else from my talk today, I want it to be this plants are not like rocks. I know they get lumped together all the time. People talk about the scenery, the rocks and the trees and the clouds are all beautiful, but actually plants are very different. They are dynamic living organisms that do super cool things.

And you just don't notice because you're not patient enough to watch them. But really they know what's going on around them, and they change based on that knowledge. Right? So a good example of this is the shade avoidance response. These plants here, two tomato plants, they're genetically identical, but if you shine far red, white on one that fools it into thinking it's in the shade, it's going to grow a lot taller, right? So this is the idea of phenotypic plasticity that plants can change what they look like based on the environment around them. This is a very different strategy than you have, because you were going to look like you look basically from the day you were born, right? The same body plant. But then if you need something, you go change your environment. You go find food, you go find water plants can't do that.

They're stuck in one single place. So instead of going to find what they need, they get really good at sensing what they have and then maximizing their growth and development so they can use the resources they have best, right? And I think this is a really cool topic and space provides an interesting place for plants to grow because there's a lot of challenges that they face there. Okay. So on earth plants sense a lot of things, right? They can sense light is the main one really important for plants. We all know about photosynthesis. Plants also can sense the quality of light. What wavelength they're getting that tells them about their position, they can sense the quantity of light they're getting, they know how intense it is. They know how long the light is, to tell them about what time of year it is.

Plants also can sense gravity, they can sense water gradients. So they know which way to grow, to get that water. They can sense nutrient gradients, they sense touch temperature, humidity, pressure, and probably tons more things. I just made this list off the top of my head. But overall they know what's going on around them, right? They have sensing mechanisms, just like you have your five senses, plants have sensing mechanisms to know the world around them. And then they respond to those changes, right? Plants respond to the world around them. They don't just know, they take action based on that information that they bring in. Now, the big question for plants is what happens when the rules change, right? So there are a set of physical rules on earth. No matter where you grow a plant, it has always had the exact, mostly same amount of gravity.

Now we want to grow them in a different place that they have never seen in their evolutionary history. And we're asking what happens? How do plants deal with this change, right? When we look at them in space, okay. Plants are going to face a bunch of challenges in space. It's not going to be a very straightforward thing for them. One of these challenges is a lack of gravity, right? This is microgravity, when you're on the space station, or in the space shuttle, something orbiting earth, that's going to be considered to be micro gravity. Because it's going to be a teeny little bit, but mostly just going to ignore that. The main thing is they lose their directional cue for growth. Gravity is really important for how plants grow. They use it to know which way is up for their shoots to grow. They use it to know which way is down for their roots to grow, right?

That's not determined by light, even though light can take over. You've all seen phototropism on your window sill, but gravity is really important. It's important for the direction that the lateral branches come off trees and the lateral roots come off the roots. Plants use it for a lot of information about their overall architecture. An indirect problem that arises when there's no gravity is there's no convection. There's no thermal buoyancy. What this means is the gases don't move around. We take that for granted on earth, that there's going to be gas exchange, the particles mill around the room that you're in. On the space station that doesn't necessarily happen. Because they just sit there without gravity, they don't mill around. This is important for plants because they rely on gas exchange a lot, right? You're familiar with photosynthesis, how it works in the leaves, right?

Plants take in CO2 and then they produce oxygen. And then we get to breed that oxygen that makes us all happy. Great. But if there's no CO2 in the immediate area of leaf and it's not moving around to get there, that's going to be a major problem. Likewise, plants also need oxygen in their roots. I know this seems weird because you think of plants that are making oxygen, but the same reason that you need oxygen to break down sugar, you use mitochondria to break down your sugar, get ATP, right? Plants do the same thing. They have the same mitochondria. It's the same biological process in their roots. They're not making any oxygen. They need to stoke that up from their environment. It's the reason that in floods, a lot of times plants will die, right? Because they basically drown. They need that oxygen.

If there is not some convection, that's going to bring new oxygen to the roots, they're not going to be able to harness the sugar energy and the cells are going to starve. There's also some water distribution problems that we see. That's the substrates that the plants grow and don't always get the same amount of wedding. But that's a little bit... It's not necessarily always the biggest problem. We also have problems with radiation in space, right? There's solar flares, other particles, these things when you're on earth, our magnetic field protects us, right? Magnetic field takes care of these things. After you get outside of that though, there going to be heating the plants causing a lot of DNA damage and this can be very stressful. There's also problems with confinement, right? There's on earth, they're always growing outside. In space, they're confined to small rooms.

There's going to be elevated CO2. You see that a lot on the space station. There's reduced pressure on the space station. There's also a problem with ventilation. So volatiles just can't get out. Sometimes these are things just from the equipment, but also things plants make themselves, right? This was an early experiment by John [inaudible 00:08:42]. They saw that, oh the seedlings, the plant seedling, this is the plant seedling that the stem was looping around. And this is because of ethylene. That's a plant hormone that was being produced and building up in a tight space, right? So they had to take mitigation steps for that. Okay. If a plant isn't growing well, how do we know what's wrong with it? Right? Because this is what we want to do. We want to say, okay, what's wrong with plants on the space station? But how do we know it's just a plant, right?

Some plants in the movies can talk to you, right? Audrey can say feed me, but really a plant's not going to be able to do that. Right? So we're going to have to figure out other ways to decide what's wrong and what they think is happening on the space station. A couple approaches for this. One approach is a straightforward one, grow plants in space, observe those plants, compare them to plants on earth and then see what happens. Right? An example of this is some research done by [inaudible 00:09:42]. And we see the growth in space is going to alter cell division and plant growth on a global scale. What you see down here, we have two seedlings, right? Here's the root down here, and the shoot up here, these were grown on the ground.

These were grown during space flight. And what you see is the space flight plants are actually a lot taller, but they also have much smaller cells. If you measure the cells, you can see, so the flight cells are here. We see about 150 cells per millimeter on the ground. You only see about 90 cells per millimeter. And you can see, these are [inaudible 00:10:19] could be images of roots. You can see in the flight samples, these cells are squished down. They're a lot smaller. And the ground samples, these cells are much larger, right? Not only are they making more cells, they're not elongating, right? There are major growth cell division cell maturation problems that can occur because of lack of gravity. This is one possible approach to answering these questions, right? We can look at these things and we can learn a lot from looking at them, but this doesn't tell us really why this is happening.

Second approach is a more molecular biology approach. For this approach, we're going to compare gene and protein levels. And we're going to compare them between plants that are growing on earth and plants that are growing in space. And then we're going to see which genes look to be important. And we're going to identify those genes function. This is basically the molecular counterpoint to the one we just talked about. And the idea here is we're going to understand how an organism reacts to a specific situation by studying the genes that uses. Okay. And before we start talking about this, we need to do some basic molecular biology. Just make sure everyone's on board with intro genetic stuff. This is the central dog mode, molecular biology. It basically says DNA gets made into RNA, which is then translated into protein.

Okay. The idea here is that all of our cells have DNA and those... and that DNA contains genes, right? These genes are then used at different times. The only difference between your skin cells and your liver cells, isn't the gene. You have the same genes in your skin cells and liver cells. It's which of those you choose to use, or those cells choose to use that make those cells different. Right? So what happens is you have your genes in your DNA when it's time to use a gene, it is transcribed into RNA. This process is called transcription. When you make RNA, and then we take that RNA and it's translated by a ribosome, into a protein. And proteins are the things that actually matter, even though they get very little of the glory, right? Proteins are the tools, they're the ones that do stuff in your cells that allow the cells to be cells and complete chemical reactions and all the things that cells need to do.

You can think of DNA as the YouTube video that teaches me how to do some home improvement project, but the proteins are actually like the hammer and the drill and the things that I use and my misguided attempt to try to fix my house right? These are things that actually have a function and not just directions for a function, which is more like the DNA. Okay. Thinking about this, we can think about what happens when we change the environment. When we change the environment, it's going to change transcription because the environment is going to say, okay, cell's going to sense it and say, okay, I need to do something new. So we're going to transcribe some new RNA and then we're going to translate that into proteins. Because of this, we can measure the differences as they happen. We can measure the difference in RNA when the cells make it.

And then we can measure the difference in proteins, protein levels when the cells make those. Right? So this is what we're talking about here. These are the techniques we're going to use to understand what a plant's thinking on a molecular level. And I'm going to tell you a little bit just for the last five or 10 minutes about some research that I did in 2015 with some collaborators. We in the BRIC-20 mission, we sent some seedlings to the international space station and then brought them back and did some transcriptomic and proteomic studies for them to see on a global level, what is changing with plants in space. The first thing we had to decide was which plant we used. Arabidopsis thaliana, all biologists should be familiar with this. It's the lab rat of the plant kingdom.

It has no agricultural value. You can't... My old advisor used to say the flowers aren't pretty, you can't eat it and you can't smoke it. However, it is a really good model organisms because it's tiny and has a sequence genome, and we can use it as a model. [inaudible 00:14:37] tell us about plants that people care about. Because 99% of its genes are also found in soybean. So we can learn about it in the small lab rat plant, and then translate that knowledge to something that people actually care about. Right? So this is a good model for the space station because it's so tiny and space is so limited in that situation. Our basic plan, first thing we had to plant seeds on earth. We planted them, seeds are like the grain of tiny, grains of sand. And we put them on petri dishes with media in them.

You can see the toothpick there for scale. These are pretty tiny petri dishes with about 700 seeds on them so we can get enough tissue. And we had to put the media in a sterile hood because you have to worry about microorganisms growing, fungi or bacteria will also really like this media. We have to make sure it's really sterile, and we also had to make sure the media was good before we actually sent it to space. So I had to make it back at home before we brought it to Kennedy Space Center for the actual experimental set up. I had to bring it on the airplane with me, who actually had to check it, right? You can't bring liquid with you. I had to check this suitcase with these four bottles of liquid that really, really looked like a bomb I imagine would look.

And I can't believe that I did not get arrested trying to put this on a plane, but it made it. I put a little note in there explaining what was going on, but I never... I don't think they ever opened it. Maybe it didn't set off the flags anyway. We planted the seeds on earth, then we put these petri dishes into this thing called the petri fixation unit. Here the petri dish slips in here, and this PDFU then fits into a brick. So six of these fit into this biological research and canisters container. We put six of them in there and there's a great doc for this on the space station that just slides right in. It's very modular and ready to go. We get those ready, they flew to the space station on the SpaceX dragon. After we get there, they germinate... We germinated the seeds on orbit by just moving them to room temperature.

The astronauts did all this stuff. I don't have any photos of it because they didn't take pictures. They grew in the dark for three days, and then they got frozen. They got put into the -80. This is an hour picture of the -80. But this is basically what it looks like. It's like a super cold freezer that you keep the samples in until they could return home. They then flew home, they had to de integrate them. This is pictures of them coming out of the freezer and taking the Petri dishes back out of those Petri Dish Fixation Units. And then the whole thing was shipped to my colleague in Ohio who was going to do the RNA extraction. And of course it came in into snowstorm and this whole giant FedEx truck just had this one tiny little box in the back.

It was very.... You could see how seriously they were taking this, right? It cost a ton of money to send something in the space. So they were very careful with the samples. We got them, this is what they looked like when the petri dishes came out. There's about, 700 little seedlings on each one of these, they're all pale because there's no white. They were grown in so they never made any chlorophyll. And then these were all scraped off of the plates and ground up. And then we extracted RNA and protein from them. And then they were subject to RNA-Seq and LCMS Ms for proteomic studies. I'm not going to get into any of the complexity of that. You don't have to freak out by the big science words. Okay. But basically I want you to understand what was going on.

Okay? Basically the idea is we have the same experiment growing on earth and in space. The same time with all the same conditions, except the space part. And we want to know there's about 27,000 genes in Arabidopsis. We want to know which of these were used by space plants, but not by earth plants and vice versa. Right? Because that's going to give us a clue to what's going on. We get the plants, scrape them off, collect the RNA from them. And we also collect some protein, then we're going to count each RNA in each protein, right? There's 27,000 possibilities for each one. We count how many of that RNA there is. Then we compare the two. And the idea here is that we're going to ignore all the genes that are the same, right? 26,000 of the genes, they didn't change between the two, we don't care about them.

What we want are the ones that are considered to be differentially expressed. Expressed in one sample and not the other. And that will give us a clue as to what's going on. We did this and what you get in the end from this is basically just like a text file with a big list of genes and the amounts of those genes and how much they've changed compared to the other genes, right? We have to take this and then make it into some interesting format to say. We had about 1,000 differences in genes and about 200 differences in the proteins that were used by the plants in the different conditions. You do not need to understand everything that's going on here. If you really want to look at this stuff, you can always come back to this paper and dive in detail. I Just want to show you is so differential expressed mRNA.

We had 968, 488 of those were up regulated on earth, which means there was more of that gene on earth than there was in space, and 480 of them there was more of those genes in space than there was on earth. And then you can look on the outside and they'll show you where those proteins end up going. You don't need to worry about any of that here. Okay. We had... There was a lot of stuff. A lot of stuff is happening in the plant, now we need to figure out what that means. To do that, we use what's called GO term enrichment. The idea behind this, is that all of the gene, not all, don't say all. Most of the genes, most of those 27,000 genes, we have some idea of what those genes are doing for the plant.

What metabolic pathways they're part of. We also know how many genes are expected to be involved in those pathways. So we found 1,000 genes and then we can say, okay, what are the chances just randomly that we'd have four genes that show up involved in the same process. And you can do wing basic stats for that and say, okay, we know that we have more genes that we would expect that show up in the negative regulation of self, a commit, right? And there's more genes that we would expect in the regulation of vitamin metabolic processes. You can see which sets of genes get turned on more than we would expect them at random. And this gives us a good clue that this is what the plant is doing to compensate for the space environment, right? On earth we have Audrey saying, feed me.

We need to have a language that we can use to read Arabidopsis right? Now we have our Arabidopsis plant and it's also talking to us. It's just telling us other things. It's saying I produce more L-ascorbate oxidase and L-ascorbate peroxidase. What that means is I'm having trouble with reactive oxygen species, right? There are reactive oxygen species that I need to quench so I've up regulated these genes. This gives us a hint on a molecular level, what's happening inside the plant. And what it thinks is going on when it's in space, and how it's responding. We can then use that information to tweak growth protocols, to try to mitigate some of the problems. Then we can also maybe target it with genetic engineering to try to fix things in that way too. Okay. Instead of getting into all this right? Which is also if you need to learn about it, you can go to the paper, super dense.

I'm just going to do a quick summary of how I think plants feel about space. The idea here is that one, I think they feel confused. We can tell this because they have some altered growth patterns, how they grow changes a little bit. We saw that they get bigger, right? They have different direction, they twist a little bit. We also know they have altered cell walls, which also are really intimately tied with growth. We know that they're stressed out. We know this in a couple ways, we see a lot of activated stress responses. The genes that are tied to responding to stress things, we see a lot of those up regulated. We also see a lot of altered translation regulation. And translation remember that's the making of proteins. And at first this was a little confusing until you think about it and say, oh, this is what plants do. when they're stressed, they can serve resources, right?

If things are going bad, you might lock everything down and only allow translation of really important things. This is another global stress mechanism. They also have some altered splicing. They change the splicing of the RNA that's going to make slightly different proteins. I don't have time to get into that now, but it's also a super cool thing that a lot of organisms perform that allows them to respond to different environmental stimuli. They also feel like it's hypoxic. This means there's not enough oxygen. Right? I touched this earlier, because we see a lot of altered metabolism and also a lot of oxidative stress responses that are going to show up when plants aren't getting the oxygen that they need. I'm out of time, I just need to thank all the people that made this possible.

One of the main ones is my collaborator Sarah Wyat. Here, her PhD, student [inaudible 00:24:08], and my master students Sarah Hutchinson, who is actually now at U of I working in the long lab and I hear that she's doing very well. They were all super helpful in getting all of this done. Also all of these people at Kennedy Space Center made getting this stuff off the ground possible. They all did tons of work. It took an entire village to make this happen. These people were all awesome and great to work with.

Also, I finally need to thank all the people here in my lab at SIUE these pictures were a little outdated because COVID, and I can't actually get new pictures of people and I didn't take Zoom pictures, but they've worked on all this stuff a lot in the lab. I also need to thank the funding sources. NASA gave us money and SIUE gave us undergraduate research money for this. And I also need to thank all the awesome people in the biology office who helped me do all the paperwork because I hate doing paperwork and they were super helpful. Thanks to all those people. And that's it. If you have questions, I would be happy to answer them.

Speaker 1 (25:10):

Yes. And we definitely like to thank you, Dr. Luesse. If anyone does have questions, you should be able to unmute yourself now. And-

Speaker 3 (25:17):

This is Josh Rovey, University of Illinois. Thank you very much for the presentation this afternoon. Question about what other, I guess, changes in the environment you identified that would cause a stress to this plant. Clearly there's microgravity, there's the hypoxic aspect, not getting maybe the oxygen that it needs, but are there other... What other stresses did you identify that would influence the plant growth?

Dr. Darron Luesse (25:48):

There's radiation, right? Radiation's a big thing we have, that's going to cause some DNA damage. That's probably the main one that's not here. There's going to be... these guys weren't photosynthesizing, because they were in the dark. But if they would've started to photosynthesize, those PDFUs like that little black box, they do not allow a lot of gas exchange. There is a little hole because you don't have a little hole in them somewhere. They're basically like a pipe bomb when you're changing pressure.

There has to be a little hole. There's some gas exchange there, but there's not a lot. They're going to be having problems with not getting enough CO_2 when they start photosynthesizing, they're going to run out of oxygen. It's also a very humid in there in that petri dish with the media and they don't necessarily like high humidity. So in older plants, that's going to lead to a problem with seed viability. I had some slides on that, but I had to cut them for time. You see seed viability issues because of humidity. The ethylene problem I did talk about. Ethylene is a gas plant hormone and when its levels build up, it's really going to mess with how they develop. All those things come together to change what the plants look like. Usually not in a good way.

Speaker 3 (27:05):

And quick follow up question. Do you measure those environmental parameters in space and then in the ground lab at the same time you're measuring the humidity, the oxygen content, the radiation exposure in both locations?

Dr. Darron Luesse (27:18):

It depends what hardware you're using. There's a bunch of different hardware you can use that are on the space station to do this thing. These bricks, the bricks that we use don't... I'm not aware of any way to just measure ethylene. That was figured out more just by seeing them like, oh, we also know that ethylene makes plants look like this. So is probably a thing we could measure temperature. All we could measure with temperature. When you do this stuff in space versus earth, there's a two day delay. You set everything up on earth two days later and then you get information back from the space station. What exactly the conditions were in the cabin, and in temperature and humidity wise and then the growth chamber where we put it at Kennedy Space Center mimics those exactly. It does its best to keep all of that static, humidity, temperature wise. It can't really control for volatiles from equipment on the space station. That could be a little bit different, but there's not much to do about that.

Speaker 3 (28:25):

Great. Thanks.