

[Theme music plays]

Hannah Senior: Welcome to this episode of The Plant Breeding Stories Podcast, where I talk to leading lights in plant breeding, asking what they do, what makes them tick and what fascinates them about the world of plants. I'm your host Hannah Senior of PBS International, world leaders in pollination control. We design and produce specialist pollination bags and tents, used by plant breeders and seed producers all around the world. And through this, I've been privileged to get a unique perspective on how plant breeding globally affects our diets, farming systems and the environment. I'm excited to share a little of this with you, as we meet some of the amazing people who make plant breeding their life's work.

Hannah Senior: Series 4 will be the last in this podcast. And it has been the most incredible experience to dig into so many different aspects of the plant breeding world and literally the world. If you're listening to this, you're part of a community that spans the globe from Albania to Zambia, Adelaide, Australia, and Ames, Iowa to Zurich.

Hannah Senior: Dr Leena Tripathi is the Principal Scientist in Plant Biotechnology at International Institute of Tropical Agriculture in Kenya where her main focus is the use of biotechnology to develop new varieties of Musa, better to most people as bananas. In this conversation we talk about why this genus is particularly susceptible to pests and diseases, making it especially important to find effective ways of developing resistance. Leena discusses how biotechnology tools have advanced this process, the many uses of bananas, and the role of regulation in relation to such an important global crop. I hope you enjoy it.

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Hannah Senior: It's great to have you hear today Leena, can you kick us off by introducing yourself?

Dr. Leena Tripathi: I'm Leena Tripathi, I'm a plant molecular biologist, and I'm the director for the Eastern Africa Hub of IITA. But I also lead the biotech research at IITA. Currently, I'm based in Dar es-Salaam in Tanzania, but I oversee the five countries in Eastern Africa, which is Tanzania, Kenya, Uganda, Madagascar, and Sudan.

Hannah Senior: Excellent. So you've got quite a wide geography under your remit.

Dr. Leena Tripathi: Yeah. And then I also lead the transgenic and the genome editing platform at IITA.

Hannah Senior: How did you come into being interested in plants, plant breeding, agriculture? Give me a little bit of the sense about your background, and maybe where you grew up, and what sparked your interest in the first place?

Dr. Leena Tripathi: I grew up in India, a city called Kanpur, which is very close to our capital, which is New Delhi, and I grew up in a big city. So I was not very close to the plant or to agriculture. I went into the pure sciences. So my undergrad is biology and chemistry. So it's botany, zoology, and chemistry, but then from there I just wanted to do something innovative. So my interest went into biotechnology and I went and I did my masters in biotechnology and molecular biology. And while I was doing my masters, my interest came more into the transgenic research, like how to develop the disease resistant or quality improvement of a crop. And mainly the concern was food security. So that's how I went into the PhD, working more on plant molecular biology, and I worked on a crop called chickpea.

Dr. Leena Tripathi: So I was the one who established the chickpea transformation in my institute there where I was doing my PhD and then, applied the transgenic approach for developing the pest resistance, using the Pt-chain. And that's where I started. Then I went and I did my postdoc in soybean, continuity, from legumes, from chickpea to the soybean, then moved on to bananas. It was very strange that my first favorite fruit when

I was a very young child, a year old or so, was bananas and I'm working on bananas. That's a really coincidence, but very nice coincidence, yeah. [she laughs]

Hannah Senior: I always think it makes it extra enjoyable if you actively enjoy consuming the crop you work on.

Dr. Leena Tripathi: Yeah.

[They both laugh]

Hannah Senior: And did you ever consider any other careers or any other applications for the subjects that interested you or did it fairly early become clear that it was going to be agriculture and plant breeding?

Dr. Leena Tripathi: From the very, very early in my childhood, I always felt like I need to do something for the human welfare and first thing, which always comes to your mind is saving life by using the medical approaches. But somehow I couldn't pursue that, and so that's when I went... But by the way, I never went into the pure agriculture. Actually, I always was on the modern agriculture. So my first degree was in science and the second one is in the biotechnology. So I didn't study agriculture.

Hannah Senior: I've heard that before, the sort of... When you're growing up, most people, unless you have a background in plant breeding, most people don't know it even exists. And so, how can children grow up saying, "I want to be a plant breeder." Or, "This is what I think is going to..." So it's interesting, isn't it? How going from that area of, I think I want to contribute to human health can then transition later on in life to plant breeding.

Dr. Leena Tripathi: Yeah, and actually I grew up in the family of engineers, so my dad was engineer. And then I grew up on a campus, which is a Engineering Institute. So, surrounding me were all engineers and my interest was more into health and then, from the health, you can relate it to the food security because, if you don't eat enough, you

can't have that good health. There a problem of the malnutrition and everything. It's quite related, yeah.

Hannah Senior: Yeah, absolutely. Absolutely. So, now in what you've described so far, you grew up in India, but now you are based in Tanzania. So, I can tell that there's at least two countries in your story, but I gather there's some more in between. So tell me a bit about how all that sort of moving around the world came about.

Dr. Leena Tripathi: I did my PhD from India, which was National Botanical Research Institute based in Lucknow in India. And after finishing my PhD, I went to U.S. and that's where I did my postdoc. So it was University of North Carolina at Greensboro. And I was still working on legumes, on soybean. And from there I joined IITA, International Institute of Tropical Agriculture. And even within IITA, I moved to several countries. So I started from Nigeria, which is the headquarters of IITA and that's where I started working on bananas from Nigeria. For a few years, I worked in Uganda, then I moved to Nairobi in Kenya and then to Dar es-Salaam, Tanzania. But I still operate both in Tanzania and Kenya because that's our hub office for the Eastern Africa, but all my research is based in Kenya. So my laboratory is based in Nairobi.

Hannah Senior: So this feels like a good place to ask you to tell us a bit about IITA. So, you mentioned it's a CG center, but could you expand on that? What's the remit? How does it fit in with the other centers? Could you tell us a little about it?

Dr. Leena Tripathi: IITA is non for profit organization based in Africa, and basically we developed the solution for the smallholder farmers in Africa for food security. And our aim is to reduce hunger, help the food security and the poverty elevation. So we do a lot of research, development, and the delivery so that the solution can reach into the hands of farmers.

Hannah Senior: And how many countries does the IITA span?

Dr. Leena Tripathi: So we are almost more than 18 countries in Sub-Saharan and Africa. We have four hubs in IITA, a West Africa hub, Central Africa, East Africa, and Southern Africa. We are part of CGIAR, but now as you might know, there used to be 15 individual CGIAR centers, but now we are transitioning into the one CGIAR, and actually one CGIAR has formally started from first, January, 2022. So there are 16 institutes which have joined and come up as one CGIAR, and IITA is one of them.

Hannah Senior: So you started off focusing on legumes, and then you transitioned into bananas. So, for most of us, certainly in the UK, most of us, first thing that will come to mind is Cavendish bananas, but of course there's more to it than that. So perhaps you could just give me a bit of an overview of the wonderful world of bananas.

Dr. Leena Tripathi: Actually, before I joined IITA, and started working on bananas for me, banana was also only dessert like a fruit, Cavendish banana, but now I know more about bananas. And so let me give you little bit history of bananas. The banana originated from two different species, *Musa acuminata* and *Musa balbisiana*. So these are like a diploid banana. So, they have seeds and they are a progenitor of bananas are grown in about 150 countries, tropical and subtropical, and some of the islands. India and China are the big producers of bananas followed by Africa. Africa produces almost one third of the banana globally produced. And even within Africa, actually East Africa around the Great Lakes region, which includes Uganda, Rwanda, Burundi, Kenya, Tanzania, and part of DR Congo. That's the major banana producing areas.

Dr. Leena Tripathi: There are hundreds of species of all the varieties, you can say, different type of varieties of banana. And there are different types of banana as well. So, there are dessert types of banana, which is like Cavendish, still in some places you can still find Gros Michel, I'll tell you why I said that, but there are other dessert bananas. Like in Africa, you will find Sukali Ndizi, which we also commonly call apple banana. These are the small bananas. They can also be eaten as fruit.

Dr. Leena Tripathi: Apart from eating them as fruit, in Africa, banana is a staple food. It's like a cooking type of bananas and they make a dish called matoke. Actually the banana varieties are also called matoke and the dish is also called matoke. The dish looks very similar to the mashed potato and is a staple for different countries like in Uganda, Rwanda, that's where I think it is a very common staple food. Then there is another type of banana which are used as roasted bananas called plantains. They are normally eaten as fried or roasted, and there are also some bananas, which are used for making beer. So they are called brewing bananas.

Hannah Senior: There you go. I never knew that.

Dr. Leena Tripathi: Yeah. So these are different types of bananas. And at some point, Gros Michel used to be a very popular dessert banana, but then it was wiped off with a disease called Fusarium wilt. And then after that, Cavendish became the most popular dessert bananas. So when you talk about the big banana growers, they still grow a lot of Cavendish. But when you come to Africa to the smallholder farmers, you will find actually many different varieties. And actually the same farmer can be growing different varieties. For their own consumption, they will grow more of the cooking types, but for selling in the market, they might grow some of the dessert type or the brewing type of bananas.

Hannah Senior: You mentioned that the progenitus species were diploid and they have seeds, but certainly the ones that are generally eaten as a dessert type banana don't have seed in them - so how does that work out?

Dr. Leena Tripathi: All the cultivated types of bananas are actually triploids so dessert types or the cooking type, they all are triploid bananas and the triploid bananas are parthenocarpic and sterile so they don't have seeds.

Hannah Senior: Oh, okay.

Dr. Leena Tripathi: Sorry, I forgot to mention that they're triploids.

Hannah Senior: No, no, no. Well, that's good. Because then that leads into questions about how does one do breeding of bananas, because if the variety that's eaten is triploid, but the breeding can't easily happen because they're sterile. So how do you go about breeding bananas? How does that work?

Dr. Leena Tripathi: In breeding, actually, they still go back to diploid. So the most of the breeding parents are actually diploids. They also have some improved parents, which are also diploids. So they cross two diploids and then they get mainly the tetraploid, and then they cross tetraploid with the improved diploid, which is an improved parent, and then they get a triploid, which is actually the cultivated ones, yeah. It's a bit of complicated. And then not all varieties are sterile, there are some varieties which can be crossed. Some cannot be crossed, but I'm not a breeder. I only know superficial part. I can tell you a little bit, but not much of the detail.

Hannah Senior: Now you mentioned the Gros Michel banana earlier - and I know that variety was effectively wiped out by disease, and yet diseases are a major problem still facing Banana growers all over the world today. Why is that?

Dr. Leena Tripathi: All bananas are actually vegetatively propagated. So they are clonally propagated. They are low in genetic diversity. That's one reason that they are more vulnerable to diseases. But the second thing I feel is the climate, because the bananas are more drawn into the tropics or subtropics and those are the places which are very good for the pest and pathogens. You will find lot of pest and pathogens, different type coexisting. So, in the same field you will find like a bacterial pathogen, fungal pathogen, plus nematodes and weevils. So with that, when pest or pathogens are there actually, they build up. So the pathogen, if it is there, it will happily keep multiplying there. So, that's another reason that you will find that once the pest pathogen is there, the control becomes a little bit difficult.

Hannah Senior: How easy is it for growers to even start their banana plants disease free? Can they get hold of clean plantlets?

Dr. Leena Tripathi: It's only a few countries where they use the tissue culture, plantlets, and then every third year they change the crop. They bring the new pathogen free tissue culture plantlets but sometimes the small holder farmers, they can't afford doing that. Commercial farmers do that, but the small holder farmers, sometimes for them, it becomes too expensive when they can get the free plantlets, either from their own field or their neighbor's field.

[Theme music plays]

Hannah Senior: You're listening to plant breed stories, brought to you by PBS International, world leaders in pollination control. We're exploring the personal stories behind people. Who've dedicated their careers to plant breeding, helping us to more productive plants, greater food security, and more sustainable agriculture. Now, back to the podcast.

[Theme music fades]

Hannah Senior: So we have then this important group of species. Important because they're consumed globally. They're very important constituents for nutritional security in certain parts of the world. And we also know we have this quite intense disease pressure. So let's just talk about that in a bit more detail. What kind of diseases or what kind of problems does that cause? And then we can go into the biotechnology and how that's being used to help tackle those problems.

Dr. Leena Tripathi: So there are a lot of diseases. There are bacterial diseases and in the bacterial diseases, there are again several bananas Xanthamonas wilt. There is a Moko disease, there is a blood disease, but the Xanthamonas wilt is quite destructive. And actually in East Africa, this disease has wiped off bananas in several fields because it spreads very fast and controlling the diseases once into the field is very difficult. So that's bacterial. There are also fungal diseases like Black Sigatoka and Fusarium wilt.

Black Sigatoka is mainly the commercial growers. They control the disease by using pesticide and spraying pesticide on bananas are not easy because banana plantation, the bananas are quite tall. So then it spraying them, you need some type of equipment. It's difficult. Then there is Fusarium wilt. So as I mentioned before, Gros Michel was completely wiped off from... Because Gros Michel used to be the most popular dessert varieties and it was wiped off because of the Fusarium wilt race one.

Dr. Leena Tripathi: And then the Cavendish was found to be resistant for Fusarium wilt race one. And then Gros Michel was replaced by Cavendish banana, but now there is a tropical race four Fusarium wilt which Cavendish are now susceptible to. So that is a biggest global challenge for banana production, I will say because it's affecting Cavendish and that's in many places, it's not only one country or two. Then there are viral diseases like banana bunchy top virus, which is also spreading pretty fast. And then there is a banana streak virus. And then apart from these pathogens, there are also nematodes, like pests, and weevils. So these are so many different types, and as I was mentioning, many of them actually coexist.

Hannah Senior: So is the main focus of your work bringing biotech to these varieties mainly focused on disease resistance?

Dr. Leena Tripathi: We are applying biotechnology mainly for the diseases and pest resistance, the biotech program at IITA. We have a very strong banana improvement program. So, we have a biotech program, but we also have a breeding program. So we tackle the problems in a very holistic way. So, when we know that this is the problem for this country, we first always look at that. Whether there are already some varieties which have resistance against these diseases or pests, because that's the easiest and the quickest way that you start recommending those varieties to the farmers. If not, then you see, okay, whether the resistance is available in the germ-plasm because banana germ-plasm. If the resistance is already present in the germ-plasm, the traditional breeding can be then applied.

Dr. Leena Tripathi: For example, for the Black Sigatoka resistance, our breeding program actually has developed some of the hybrid varieties using the conventional breeding. But then when we are handling a disease where we know that there is no natural resistance is available in the germ-plasm, that's when then the biotechnology comes into play. And when we talk about biotechnology, now we are applying transgenic approaches and we are also using the gene editing. Transgenic approaches is mainly when we are bringing the resistance genes outside of bananas, but then gene editing comes handy or I will say is more powerful when we have already some information maybe from the wild type progenitor. And then we want to actually use that information to develop the resistance into the cultivated susceptible varieties. Then gene editing is very powerful in doing that.

Hannah Senior: And are you focussing on just the big disease threats or are you trying to bring in resistance to lots of biotic threats at the same time?

Dr. Leena Tripathi: Overall goal is actually to have the multiple disease resistance, because as I was keep on emphasizing that some of the diseases and pests are actually coexisting. So, if you develop a resistance to one disease and the same variety is susceptible to a second disease and is the disease... That pathogen is also present there, releasing that variety doesn't help. So in parallel, we are actually trying to develop the technology for different diseases and pests, but our ultimate goal is that if we can actually stack them so that we have the varieties which have resistance to more than one disease and pest.

Hannah Senior: And if you're looking to the existing varieties for resistance, where do you get your germplasm from?

Dr. Leena Tripathi: So we do have that gene bank at IITA, at our headquarters. So, we have lot of collection there, but we don't work in isolation. We work in partnership, right? We have collaboration in India, they have a big collection of the wild type bananas. So we know each week through our collaboration, which we can work on, East Africa has

lot of banana germ-plasm, which we can always step on. So, we can access the germ-plasm, yeah.

Hannah Senior: Could you give an example of a problem that you have managed to address using biotechnology tools?

Dr. Leena Tripathi: Let me give you the example of banana Xanthamonas wilt. In brief, we call it BXW. So this disease was initially reported on Ensete, that's a crop which is closely related to banana in Ethiopia. So this disease was there for five decades, but for long time, it was actually confined to that crop and also Ethiopia. Then from Ensete, it jumped to banana, but it's still quite confined to Ethiopia. But then in 2001, this disease was actually reported in Uganda. And after that, it has spread to other countries like Kenya, Tanzania, Rwanda, Burundi, and DRC. And these are the countries which are the major banana producing areas.

Dr. Leena Tripathi: So it has a big economic impact. This disease has caused, over the decade there's estimate is like two to 8 billion dollars losses because of this disease. But more than that, I have visited a few farmers where I have seen that the whole field was wiped off because of this disease. Because once this bacterial pathogen is there, it actually spreads very fast and then there is no other remedy than to uproot the banana plant. So it leads to the actually a hundred percent loss and the disease is spread through insects, and also through the trading material.

Dr. Leena Tripathi: There is no resistance in any of the cultivated varieties against this disease. So we started looking at what can be done. So, we started several things in parallel, like screening of the germ-plasm, but also seeing if we can start developing the transgenic. The resistance we found was only in the wild type. One of the wild type banana called *Musa balbisiana*. And *Musa balbisiana* is not commonly used in the breeding programs, because it has another problem of the streak virus. So in the starting through the literature search, we found some genes, which we thought can have potential to develop the resistance against this disease.

Dr. Leena Tripathi: We tested several of them, and two of them, one is called plant ferredoxin-like protein, PFLP, and the hypersensitive assistant protein, HRAP. We actually tested those genes and we got very promising results. So these transgenic bananas, we tested under the confined field trials in Uganda, and they showed complete resistance against these disease for the successive crops. So like the mother crop, but also the return crop. So we tested several generations of the crop. The performance of these transgenic bananas were actually similar to the control non-transgenic bananas, which are very important, because we tested the yield and other aspects. After that, because the single gene based resistance can break down very easily because the pathogen mutates very first. So we have actually stacked the two genes together. And so we are right now on the product development, because we tested them in Sukali Ndizi, but now we are transferring that technology to the Matoke varieties, which are mainly grown in East Africa. But that's one of the successful project I will say because we have proven that technology is working, not in the glasshouse, but in the field for several years.

Hannah Senior: Because it's transgenic are there any obstacles or barriers you face when it comes to releasing it?

Dr. Leena Tripathi: Yeah. So, not all the countries have the biosafety regulation in place so that you need those regulations in place before you can release the variety. Most of the countries where we are targeting where the disease is present is very difficult. So few years back, you remember I mentioned that the wild type banana *Musa balbisiana* has resistance to this disease. I thought, let's study this wild type banana and find out the molecular basis of the resistance for this disease. So we did that and we got very good information. Now we have the whole idea, like which gene goes up regulated during the pathogen infection, which goes down regulate. So based on that information, we are now editing the banana. So we are not putting foreign gene or anything. We are trying to tweak the endogenous banana genes so that there is no foreign gene integration in them. And then once we are ready, those products will be non-GMOs. So

that's the main focus of our group right now. So we are working on gene editing, CRISPR/CAS mediated gene editing, like knocking out of the susceptibility genes.

Hannah Senior: And are the biosafety regulations that you're facing just in relation to transgenic varieties or also for gene edited ones too?

Dr. Leena Tripathi: It depends which country you're talking about. There are several countries where the gene editing product, if there is no foreign integration in them, they are not regulated as GMOs. Those are the, like in U.S., Canada, Australia, Japan, and then several other in Latin America. But then when we come to Africa, Nigeria is the first country which has developed the regulation last year. They have now the guideline, which also says that if there is no foreign gene integration in the gene edited product, then the product will not be regulated as GMO. So there is a difference. And then Kenya is actually at the very advanced level of approving. So they have a draft guideline. We are waiting for the final approval.

Hannah Senior: So asides from your breeding programs, what other challenges do you face?

Dr. Leena Tripathi: So recently, we also started working on Fusarium wilt, as I said, that this is also becoming a global challenge now because of the TR4. So we started working on Cavendish as well. So now in our lab, but we are at the very, very preliminary stages. So far, I was more talking about at the higher level, how we do the work, but when you go technically more details, it's not that easy. Banana is a bit difficult crop to work on. So we have to spend few years in developing cell suspensions and also right now, what we are trying to do is, we are trying to see how we can integrate gene editing into the breeding program. And the second one is we also want to improve the parent for the breeding program, so the gene editing can be more integrated into the breeding program. So we want to use the bio technology to complement the challenges which the breeders are having.

Hannah Senior: So, do you anticipate that the varieties that you are working on and the resistance that you're introducing will then be capable of being used globally? Is that part of the vision?

Dr. Leena Tripathi: If it develops the Cavendish resistance to these ones, this can be used globally. It's not only for the African countries.

Hannah Senior: So when you think back over your career, we've talked a lot about bananas and we didn't touch particularly on challenges that you faced before coming to this role, but you must have had ups and downs. So tell me about a time when you've had faced a real challenge and how you've got through that, how you've kept moving forwards?

Dr. Leena Tripathi: Is one challenge is actually the funding, because we are non for profit institution and we are project based institution. So, apart from focusing on the research, we have to also keep on looking for the funds, and then when the project is coming to end, and if you don't get the next phase, so what to do, whether that technology goes on the shelf or how to continue that one. And that's a real challenge, because some of our technologies which we have developed on the project actually are sitting on the shelf because the lack of funding. And then, the second challenge is definitely the regulatory side. It's a time consuming thing to get all the approvals and all those things. We haven't reached to the stage where the product is ready for the commercialization, but we know that yes, that is not easy.

Hannah Senior: So what kind of opportunities do you see for the future? What are you excited about?

Dr. Leena Tripathi: I see there is a lot of opportunities, particularly for gene editing that technology for its application. This is a really powerful technology, and I will not consider gene editing as just a standalone, that technology can solve all the problem. I'm not talking about that, but that's a powerful tool in a toolbox. And I also see a little bit

change in the mindset of people. So, people who were more against the GM, because they have a lot of fear. I think whenever there is a new technology, it's natural to have a fear against that. So now, scientists and other people have so much experience that if you bring a new technology, you also know that you have to start public awareness early enough. So with that combination, I really, really see a bright future for biotechnology as a complimentary to the breeding and to the other technologies. Is still, there is what is going on in Europe, influences other places.

Hannah Senior: Tell me more about that.

Dr. Leena Tripathi: When a layman hear that in one country, they are talking against of this technology, this person always sit back and feel like, "Oh, why?" That's what we, not only me, but it means many people, we are trying to provide the scientific evidences to do the general awareness to say that no, this technology is not bad. But I see at least in the genome editing, many countries are coming up. For example, Japan has approved tomato, which is helping other countries.

Hannah Senior: Yeah. I suppose it does take time for fears... Well, it's two things. It takes time for the evidence to accumulate so that fears can be allayed. And it also takes time for the debate to sort of play out in the public arena, for both sides to get their story across and for a consensus to begin to form about how could these tools be used or where are they best applied, those kinds of things.

Dr. Leena Tripathi: Yeah, because it's several time I go in the meetings and then people do ask me, "Oh, but why you are all consider gene editing products similar to the GMOs? What's the concern?" So, you keep on repeatedly those type of questions. So, you can't say that what is happening in Europe doesn't influence other places. It does.

Hannah Senior: Yeah, that's interesting. Well, I have learned so much about the Musa genus that I didn't know before and it has been such an interesting conversation. Thank very much for sharing you story with us, Dr. Leena Tripathi

Dr. Leena Tripathi: Thanks. Thanks, Hannah.

[Theme music plays]

Hannah Senior: You've been listening to Plant Breeding Stories by PBS International, and I'm your host Hannah Senior. Plant Breeding is a pretty specialist podcast topic, which can make it difficult for people who share our interest in this kind of thing to find it. So if you've enjoyed the podcast, recommend it to your friends and colleagues, and please help others in the plant science community to find it by rating this episode and subscribing to the series. You can contact me on Twitter at PBS Int., or on Instagram at PBS underscore Int. Until next time. Stay well.

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