**ACPPO WEBINAR - Grains Research and Development Corporation visiting fellow- Dr Thorsten Langner**

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Thorsten leads an independent research group focusing on adaptive evolution of plant pathogens.
His group is mainly interested in adaption of genomic variants of the fungal pathogen, commonly known as wheat blast.
This research also involves plant pathogen surveillance, molecular evolution research, and it aims to translate the knowledge into disease management strategies through gene editing.
So I'm now going to handover to Ken Young, the senior manager for biosecurity and regulation at GRDC, who will briefly talk about the biosecurity risk of wheat blast and the significance of this research in this area. Thanks, Kev.

Kevin Young:
I'd like to welcome Thorsten Langner to Canberra, but also to Australia, so as we were told, Thorston is part of our visiting fellow program,
In the interest of Australian researchers, but also the grains industry, to gather the best experience and bring across Australia to share their message.
So Thorsten is one of our first visiting fellows. He spent some time at the Centre of Crop Science in Perth, at Curtin University, and also was there for the international symposium.
So it's excellent that he has also come across to the east and is going to interact with some researchers here, so very much welcome.
So let me just quickly switch to wheat blast, an exotic disease for us in Australia.
It's moving more recently around the world, which has caused us some concern.
So knowing how we might be able to manage it if we do get an incursion.
Recent modelling does show wheat blast has the potential to impact our grain crops and our wheat crops, especially in the high humidity areas.
So that’s both the North which has potential warm summers and also warmer winters down in Tasmania too.
It's very much of interest to hear from Thorsten, welcome.

Thorsten Langner:
Thank you very much everyone for joining.
Yeah, so it's my pleasure to be here and talk. And yeah, so I'm going to talk about today my group.
I just kind of started my independent group at the Max Planck Institute.
And in my group, we are really focusing on like different aspects of plant pathogen evolution.
So we want to understand how plant pathogens can adapt to new environments, but also to new host plants. And we're doing that at different levels ranging from genome evolution and kind of like how genome evolutionary events determine sort of like the molecular evolution of virulence related proteins. And then we can use this information to study the function of these virulence related proteins.
So these are usually called effector proteins.
And we do this mainly from like the standpoint of effector host target integration. And ultimately we try to integrate this information to inform disease management and on the molecular level, we are currently following more of a bio-engineering approach that we talk a little bit about later in my talk.
So we are studying adaptive evolution of filamentous plant pathogens.
So mainly focusing on fungal diseases using genomics.
So we studied genome evolution and the influence of genome evolution on the molecular evolution of virulence related proteins, and how these molecular evolutionary events determine the function of these products in the context of effective wastage.
Ultimately, we can use this information to inform disease management.
But we can also use this information for new approaches to develop immunity. That's what I'm going to talk a little bit about at the end of my talk.
So if we look at pathogen surveillance or general pathogen evolution, we have to keep in mind that pathogen genomes are usually compartmentalized or organized in a certain way.
Many plant pathogens - this kind of is visible in the genome through regions that are relatively gene dense and regions that are relatively gene poor but very rich in repetitive and transposable elements.
And this kind of genome structure has led to the two speed genome model, which basically says that fast evolving genes that are usually related to virulence are located in these regions of kind of low gene density, but repetitive element richness.
So this is true for a lot of pathogens, not for all of them.
So we have different types of genome organization. For example, in the blast fungus we see this type of heterogeneous genome organization. And so this pretty much follows this two speed model of genome organization. And in the blast fungus we specifically see kind of an enrichment of repetitive elements towards the end of chromosomes, where we also find virulence related genes.
Of course, we can have epigenetic compartmentalization.
Sometimes called A and B compartments, but eu generally refers to hetero and euchromatic compartments that are maintained either through stolen modifications or through DNA methylation. And what we have been mainly focusing on in the like recent years is genomic compartmentalization in form of extra chromosomes, so in the blast fungus, we see these supernumerary chromosomes, or in the blast fungus, they are usually called mini chromosomes and they can facilitate genetic diversity by basically mediating breakage fusion cycles in the genome so they can facilitate the emergence of new structured variants, but can also lead to copy number variations or present substance variation. And we see that these chromosomes follow mainly the kind of two speed genome arch.
These extra chromosomes are usually repeat rich relative to the gene pool, but they can harbor variance effector proteins.
And over the past few years, we've seen that those chromosomes can contribute to new structural variants in the genomes, by recombination events that happen between those many chromosomes, but also within or between mini chromosomes.
And this can basically lead to the emergence of entirely new chromosomes in pathogen populations.
And in addition to this, we recently found evidence for horizontal gene transfer and this is in form of the transfer of entire mini chromosomes between pathogens and all these mechanisms can obviously increase the genetic diversity and can increase the adaptive potential of a pathogen population.
We can use these patterns to inform disease surveillance or diagnostics not only because over the years, genomics has contributed to better understanding of concepts in plant pathology, such as the gene for gene concept, but it has also shown us that there's actually more complexity to this where ambulance genes are often present in kind of a whole set of allelic variants and they co evolve with variants of the blood immune receptor, which are usually kind of co diversified, these pathogen effectors.
We also observed very high levels of intraspecies diversity in most pathogens and this can come in the form of present absence variation where certain genes or regions of the genome are only present in some of the isolates, but can also come in form of copy number variations and this is important. We talk about genetic resistance that you are able to use proteins for disease management.
But this intraspecies diversity can also come in other forms, for example, in some dikaryotic fungal, we see patterns of hybridization to contribute to genetic diversity, and I think in recent years it's becoming more and more clear that horizontal gene transfer has a contribution to increasing genetic diversity and pathogen populations. And all these patterns together can be used to infer the population structure of plant pathogens, but can also be useful in determining migration patterns of pathogen populations.
So basically we can use all these patterns for genomic surveillance at different levels, going from single gene level for example like through PCR.
It is like quick lamp based, usually a letter flow test all the way up to kind of studying meta populations by meta genomics.
So we are doing this in the blast fungus magnaporthe oryzae and this fungus is probably mainly known as the rice blast fungus, but in recent years weed blast has become a little bit more of a concern.
But species of magnaporthe can really infect all cultivated crops, ranging from wheat and rice to different types of millets, oats, barley, you name it.
And in addition to cultivated crops, this fungus can also infect wild species and actually over 50 wild breed species.
And these wildcress isolates are also important for us to understand the evolution of the pathogen population and this is because we see a very strong association of specific genetic lineages with certain host plants.
So what you can see here is a phylogeny of the whole species of the blast fungus.
Where certain genetic lineages are usually associated with one predominant host, so there's certain degree of host specialisation which basically leads to these patterns of incipient speciation of basically like this is reminiscent of kind of new species emerging and these pathogen lineages evolve largely independent of each other, so that means that we have different levels of genetic diversity here, ranging from kind of the whole species diversity that encompasses all these genetic lineages, but all the way down to kind of, you know, very little genetic diversity in species host adaptive and so today I'm talking a little bit about the rice infecting lineage and afterwards about the wheat infecting lineage.
So we were interested in adding structural genomic variations to our kind of understanding of pathogen evolution and to do so, we are using the rice infecting lineage at the moment to understand what the effect of structural variation is on the kind of phenotypes that we observe in pathogen populations and to do so we want to limit the sort of like background genetic diversity as much as possible to really focus on the phenotypic effects of structural variance.
And we were actually quite surprised by studying a local population here from northern Italy and in Europe we only have a single clonal lineage of the rice blast present. So this is an asexual lineage.
There's only one mating type present, very limited potential to increase genetic diversity in this population, and we see that at least over the last 150 years this population has remained largely homogeneous.
But if we look at the structure of the genome here, so this is a shift trail where we separate entire chromosomes.
Then we see that there's actually much more diversity than expected present on the chromosome level here at the lower half, you see this variable extra chromosomes where we have some chromosomes that are more conserved, so they are present in the majority of the isolates.
But then we also see very unique chromosome contents in specific isolates and we developed a method that allows us to sequence all of these chromosome separately from the whole genome assemblies and this really allows us kind of to submit and study biology of these extra chromosomes in our population, and I just want to mention one aspect of this project where we see that these mini chromosomes can almost act like mega transposons in the genome of this fungus.
So what we see here is just kind of sections of whole genome alignments and at the top is the reference genome for rice blast.
And this isolate doesn't have any mini chromosomes, so it only has seven core chromosomes.
But you see that some of these core chromosomes can share sequence content with those, and then we see that kind of structured variation present in these many chromosomes.
So for example, here in the form of this inversion here. But in general, these many chromosomes can be relatively conserved in the population.
So then if we look at the bottom here, we see that some of these isolates contain this mini chromosome, but here it has rearranged with chromosome. So basically what we are seeing here is a chromosome fusion that happened between the mini chromosome and the chromosome.
And this led to almost a fusion of the entire mini chromosome. But we see here that roughly 100 kilobases of sequence content gets lost in the process.
And this is obviously important if we think about the kind of adaptation of pathogens, especially to react to genetic resistance, because this can obviously lead to a loss of ability in the pathogen population. And interestingly, what we see in this isolate is when we combine the mini chromosome data and the whole genome.
And the physical separation of these chromosomes here that during the evolution of this isolate, we actually must have had a chromosome triplication where one copy of this chromosome gets maintained as a mini chromosome in the population.
Then we have another version of this mini chromosome that is kind of a smaller, degraded version of the same chromosome, and then an additional copy has integrated into chromosome, generating new structural variants.
So essentially this mini chromosomes can contribute to present absence variation to copy number variation and can actually change the genomic context of the sequence chromosomes.
So I just want to kind of break this story down to the essentials.
So we're dealing usually with clonal images, and clonal images are responsible for most of the epidemic outbreaks in most regions of the world, and intrinsically they have very large diversity. But through the formation of these extra chromosomes, we can basically generate new genetic variants and by recombination, they can change the sequence context in pathogen populations.
And in addition, we recently published the first observed horizontal transfer event in in magnaporthe of such a chromosome, and together all these mechanisms can increase the genetic diversity even in asexual clonal populations of this disease and ultimately leads to increased evolutionary potential in these pathogen populations.
And importantly, obviously we found that wild grass infecting pathogen populations can actually serve as a donor of genetic material and can lead to the acquisition of new genetic material in crop infecting pathogen populations and that kind of brings me to my first sort of main message, which is that we really should embrace the concept of zoonoses a little bit more in plant pathogen evolution.
So this is very common in surveillance of human pathogenic organisms where host jumps from wild animals into humans are very well documented, but in plant pathology this is still a little bit neglected and we really have to integrate a little bit of ecological understanding of Y pathogen populations to really understand the evolutionary potential of crop infecting litigants. And this is also important, because Y pathogen populations can actually serve as AI.
Think early warning system, so if we think about pathogen surveillance, then we usually focus on cultivated fields, but obviously cultivated fields are very well maintained and usually we spray fungicides. So we actually actively suppress the emergence of new diseases and so cultivated fields are not necessarily the best indicator if the conditions are right for a certain disease.
And we've seen that recently by looking at white grasses in Germany. I really found these plants by chance, this was in 21 and so here you see quite heavily infected certaria plants.
So these are wild Millet plants and this is not, you know, kind of a mild infection.
So this is really a quite heavy infection here and in Europe plant diseases are usually thought to be kind of contained in the Mediterranean and the climate in central Europe is thought to be non-conductive for the disease.
But obviously we found this disease on wild plants, which indicates that the climate conditions might already be suitable, at least in some seasons.
Like for this disease, and this is obviously concerning here because we found this disease here in Southwest Germany, which is pretty much right in the centre of the Central European cereal belt.
So this is kind of the area where most of the Barley and wheat is produced in central Europe.
So what we achieved by looking at this wheat population?
This is basically that we found the Northern most occurrence of blast disease worldwide actually in a zone where it's not supposed to occur.
So wheat plants can really observe detection system to determine if the climate of the conditions in a certain regions are suitable for a certain disease before we actually observe it in cultivated fields.
OK.
So now I want to jump to wheat blast a little bit.
We heard already that wheat blast is becoming more concerned globally.
And this is because wheat blast is a very young disease.
It's only 40 years old and was pretty well contained in South America, so it has spread through South America, but it hasn't escaped from South America.
But recently this disease occurred in Bangladesh and Asia and in Africa and this is a photo taken in South America as you see here the bleaching of these heads, which is a very common symptom for wheat blast and in the right conditions, you can see that this disease can pretty much completely eradicate the harvest.
And this is a problem not only because obviously it's a very serious disease, but in South America, for example, we see a fungicide resistance is increasing. New genetic variants popping up and overall, the disease seems to become a bit more virulent than the original isolates that belong to this founder population.
So I mentioned already the young disease, but in South America it has diversified into quite a genetically diverse population.
So we see certainly different sub lineages of wheat blast present in South America, but probably even more concerning, over the years we see that the population structure seems to change and tends to go towards a single genetic lineage and this is what we call the pandemic lineage.
So this is a lineage that is slowly taking over South America, but this is also the lineage that has spread to both Bangladesh and Zambia, and we analysed isolates from both of these occurrences and we can basically conclude that they all belong to a single clonal lineage that seems to be very, very effective in spreading at the moment.
So following the outbreak in Bangladesh in 2016, we started, well If I say we I should say Sophine Kamoun Slat is my former supervisor. Started this open wheat blast initiative and the goal here was really to release data as fast as possible with kind of as little delay or bureaucracy involved and what we used here was a field pathogenomics approach.
So basically we received infected plant samples from Bangladesh.
Are indeed RNA sequencing directly on these lesions, and this really allowed us to move very quickly.
So we didn't need to cultivate the isolates, we could directly analyse genetic identity of these isolates and that led to a very rapid identification of the lineage.
Received samples in February 2016, we had the data generated six weeks later. So six weeks after the outbreak, actually.
And the first preliminary analysis were done basically two to three months after the outbreak. And you see here in these articles that other groups actually joined our activities.
So we started kind of making this home page where we can release our raw data sets and then groups from France and from Switzerland actually conducted independent analysis to identify the lineage of this pathogen and this led to these two -
another one down in Switzerland and both of these analysis independently concluded that the wheat blast isolates collected in Bangladesh are very closely related to the South American isolates here and most likely pointed towards Brazil, but yeah, obviously we don't have absolute certainty.
So we can only tell that like those isolates came from South America.
And yeah, following this analysis, we kind of received the concerning news that two years later there was a second incursion that led to the transmission of wheat blast to Zambia.
And this time we were like already a little bit further advanced in our diagnostic approach.
So this time we use a short amplicon sequencing based approach and this really allowed us to scale up our analysis to many, many more samples.
So this time we were actually able to use short amplicon sequencing and scale up our analysis to all isolates that have ever been sequenced, plus all the new isolates that we receive from Zambia and the isolates we had from Bangladesh.
So this is now really kind of enabled us to expand the diagnostic space, which was usually limited here, to this whole genome assembly isolates - to many, many more isolates by just reducing the complexity of our diagnosis.
And the way we did this is by kind of designing a diagnostic snip panel of only 84 snips and we use these diagnostic sites for short applicon sequencing and this is enough to get a very good separation of pathogen images. And in this case, we specifically design the diagnostic sites to differentiate different wheat blast lineages so you can see here wheat blast in bloom.
So we get a very good separation of the South American lineage here and we could basically conclude that all isolates belong to the same clonal lineage which we call this B71 cluster, based on the name of the reference genome for Wheat Blast, which is B71.
So this really shows that the exact same initial transmitted twice actually, and we could conclude from this study that it was actually 2 incursions and both were kind of transmission events from South America. So independent transmissions from South America to both Bangladesh and to Zambia.
So like this kind of scalable approach really allows us to have very rapid genomic surveillance and identify variants of concerns in pathogen populations very, very quickly and at scale, and we can then actually use this information that we get from genomic surveillance for confirmation of these variants in the lab. We can do that either by genetics or through phenotyping and combined this information to feed it back into a pathogen informed disease management strategy.
And this can be for example based on presence or absence of virulence genes.
We can analyse fungicide resistance so we can basically include any information that we interested in, in this diagnostics.
So another observation that we made while analysing these new isolates was that all of these pandemic wheat blast isolates actually containing extra chromosome that was not present in the original founder population in South America. And interestingly the presence of this mini chromosome in this modern isolates is also associated with an increase in virulence.
So we are currently hypothesising that copy number variations that are associated with the presence of this mini chromosome, contribute to this increased virulence.
And we're actually trying to counteract this by basically selecting against this chromosome. So why we can do that is by targeting specific virulence genes.
So we identified 19 effector candidates on this chromosome.
And these will include some very well known effector proteins that have been studied since 30 years.
But we also find new effective genes on this chromosome, and interestingly, one of these candidates we've been working on, in the context of rice blast already and just before I get to the data, we just have to have a brief look.
What effect does it affect the process into the plant cell where they can interact with plant proteins? We just call them effector targets here, and sometimes this interaction leads to suppression of immune responses.
So suppression of plant immune system, which can then ultimately cause disease.
But on the other hand, intracellular effector proteins can also, be recognized by intracellular immune receptors.
We're mainly focusing on these coil type NLR’s, so they have this internal, a central nucleotide binding domain and these C terminal loose repeat proteins and from this triangular kind of like interaction pattern in the host we can kind of derive like a few common concepts in path evolution and the first one is that effector always have to adapt to evade immunity.
So if an effector protein or the action of an effective protein is recognized by the plant immune system, this strong selection pressure ultimately leads to the emergence of what we call stealthy, effective variants - these are effectors that can still fulfill their function, but they are not recognized by the plant immune system, but it can also lead to deletions or silencing of A variant strains, so this is very well studied, but then on the other hand, Pathogen effectors also have to adapt to new host targets, so host targets that are attacked by pathogen effectors usually diversify quite rapidly as well.
And this can lead to loss of function, so pathogen effectors are continuously have to adapt to these new target variants or in the context of blast disease which is a multi host pathogen. They might have to adapt to completely new host targets.
And the third concept, I just want to briefly introduce is, that some of these effector target domains in evolutionary times have integrated into the blind immune system where they're now acting as integrated domains and still have the function to bind effector proteins.
But basically what the plant achieved by doing this is to turn the susceptibility gene into a resistance gene.
And this is pretty much the concept that we want to exploit in our approach to generate wheat blast resistance.
So we basically want to kind of turn weak points in the plant into an immune response.
The way we are doing this, so we have some proof of concept for rice blast already.
So we've identified the target proteins of the effector PWA2 and you see PWA2 is also one of the effectors that are present in wheat blast especially have increased copy numbers in this pandemic lineage of the wheat blast.
And we know that this effect of binds to heavy metal associated domains in rice.
And you see that these indirections are quite specific.
So we screened a whole bunch of heavy metals associated proteins.
But only one of them actually binds to PWA2 and we can confirm this by purifying these proteins. And like in this case we used either thermal titration to basically show that this effector index with the host target in a one to one ratio. So one molecule of defector index with one molecule of the host target.
So now we can use this information for a bioengineering approach, so we can take an immune receptor that contains an integrated domain and we can replace this integrated domain with an effector target basically.
So what we do here is we just replace this like internal domain here and what we achieve by this here on the right side you see kind of transient assay that we use in nicotinab and tamiana to see if an immune receptor can elicit an IMM response. So what you see here is cell death, which is the immune response. And here in the wild type receptor, this causes immune response with the cognate effector ABR15.
So now if we look at the bioengineered version of this immune receptor, we see that we can actually switch around the recognition specificity.
And now we have an immune receptor that responds to PWL 2.
So now how can we use this for wheat blast?
Recently we have also identified the wheat arthro immune receptor which we call TA pick.
Interestingly, it contains integrated HMA domain at the exact same position as the rice mute receptor, and we went ahead and confirmed that this immune receptor is functional and we can actually use it in our transient to screen for an immune response, which now allows us kind of to do domain replacement domain engineering to target pandemic plus isolates.
And I just got this result sent by my PhD student, kind of couple of weeks ago.
So it's very preliminary, but what you see here is a comparison between the rising receptor on the left side of these leaves, the wheat immune receptor on the right side of these leaves and on the top, we have the white type version and on the bottom we have the Biome engineered version, so here we can see that there's an immune response like a mild immune response caused the system by combining the rising immune receptor with effector ABR pick.
But we don't have an immune response to PWL 2.
And the same is true here on the right side, where we get a very weak response when we use the wheat immune receptor in combination with API pick.
So this not necessary, they don't necessarily have to co-evolved, but we still get a very weak immune response.
But you see, like when we when we engineer this immune receptor, we can get a very, very strong immune response to PWA 2 using the rice immune receptor and we can also gain recognition of PWL2 using the wheat immune receptor scaffold, so currently we're trying to boost this immune response.
Interested in understanding how paired and how paired immune receptors can cooperate together to actually give a strong immune response and we are working more on the engineering of these HMA domains to well basically boost this immune response further.
But essentially, we're having kind of the first step here for an engineered resistance against wheat blast.
And with that, I'm at the end of my talk.
These are all the people who were involved in the project.
And yeah, thank you for your attention and I'm very happy to take questions.
So does anybody have any questions now?

 **Kilmartin, Charlie** 35:28
Just hearing about the innovation to turn some of the receptors into defenders.
Basically right, what are the challenges you're facing there to get that right?

 **ACT CQ2 02.022 Tiger Orchid Room (VC Unit Type 1 with IPTV)** 35:40
Oh yeah. So one of the most common problems, I think with any immune receptor engineering is that we often get auto activity.
So often these domain replacements just cause like an autoimmune phenotype.
For the rice receptor, we have a few strategies. How we can mitigate this. So we identified a few locations in the immune receptor that when mutated we can reduce auto activity.
And we can use aligns mismatching to reduce order activity.
So basically, if we combine different alleles of the sensor and LR with the helper and LR, then we can reduce order activity phenotype. So this is one of the most common challenges.
Another one is obviously it's not always easy to really gain noble recognition specificities.
But you're drawing on the diversity of target families.
We can like relatively easily overcome that.
One of the fundamental gaps in our knowledge is really that binding.
So, like we can obviously engineer binding relatively easily, but binding doesn't always translate into recognition even in our transient systems and we don't really understand why yet. And even if we have recognition in transient systems doesn't necessarily mean that we get resistance in the wheat.
And again, we don't really know what the molecular reasons are, why this doesn't always translate.
Sometimes it does, sometimes it does not, and we don't know why.
And then obviously like in the later stages when we generate the first proof of concept, probably transgenic plants, but ultimately we can do this through infra genesis.
Then the next challenge would be to target the right tissue.
So not all immune receptors are previously expressed in plants and for wheat blast. Obviously it's going to be important kind of to get expression in the heading stage.
But yeah, this can probably be done through promoter engineering at the later stages.
So currently we're focusing really in establishing the framework of immune receptor bioengineering. And once we once we go into the plant, then we probably have to shift our focus more towards a more physiologic engineering approach.

Damien's got a question about can this approach be applied to other wheat diseases or the approach into other crops?

Yeah, I think so.
I think at least based on our current experience, we can engineer binding to almost anything.
There are different examples and different degrees of sort of like artificial immune receptor engineering.
So we are obviously using kind of a system that draws on natural variation of effector targets, but in Soufin's lab they have actually engineered resistance against GFP, so the print fluorescent protein. So what they used is actually a nano body.
So these are antibodies produced in Camelid animals and like the unique aspect of these antibodies is that they are single chain antibodies.
So you can kind of integrate them so they use these antibodies as interpretive domains to engineer resistance against GFP against RFP and they are currently kind of screening antibodies against a range of pathogen effectors.
So ultimately, we can pretty much use synthetic design of proteins to target any pathogen. Obviously with this kind of thing, we're facing different issues of consumer acceptance.
But theoretically this is possible.
We are focusing on intrinsic plant domains mainly because, yeah, this is, most likely the next step that gets deregulated in the genome editing space, which will allow us to bring this to the field.

Thank you.
So Brendan Reading is online from the Victorian government.
It's a great talk, and with the surveillance the 84 SNP chip are the 84 SNPs located across the genome or localised on the chromosome?

Yeah. So some of them are somewhat clustered. So they are not completely evenly distributed. And this is mainly because the diagnostic regions across chromosomes are not equally good.
So we have kind of, we have blocks of blocks of SNPs that are kind of somewhat clustered, but these blocks are still spread out over the genome, but essentially you could design the snip panel pretty much as you like.
So like in our approach, we focused on the regions that are most diagnostic to separate V plus images.
But essentially we can screen for anything - we could include short amplicons to determine the meeting time.
We can include snips that determine specific fungicide resistant snips.
We can look for specific genes, so there's really no limit.
Is probably the number of snips that we can do in one go because so the way this method works is basically you have hundreds of PCR reactions running at the same time and then you use the output of these PCR reactions here for sequencing.
So basically we have one pot reaction where we do in this case of 84 PCR reactions.
And then we feed this into sequencing.
Yeah, but this can be designed depending on yeah, what you want to look for.

Thank you.

So Andy Shepherd from CSIRO is in the room.
So you told us about how this wheat blast is quite a generalist pathogen globally, but it's evolved.
You show that nice phylogenetic tree of how it's evolving into particular lineages of tackling particular host groups.
Is that evolution quite stable or is there indication that a strain within one of those planes could leap in and start and diversify back into other host.

Obviously, the number of strains that are getting around the world is limited compared to those in South America and therefore how much we have to be worried about the ones that might be getting close to Australia.
So like first to address your first question.
In the wheat blast lineage, at least from the information that we have so far, we obviously have much more genetic diversity than other lineages and we don't fully understand why that is so obviously weak plus is very young and it seems that there was a period of recombination that might have contributed to genetic diversity.
And you see, like here on the left side of the tree, kind of the different colours in the branches.
So like this indicates kind of the host plots that those isolates we’re setting were collected from, so in the weedplus image and in the white perennial rightcrest infecting village, there is certainly kind of some potential for, you know, ongoing host jumps, we don't have very good data on the potential of host jumps in general in order lineages.
And again, this comes back to that we have very limited data from White House populations, so like White House, usually the main candidates for host jumps and yeah, we just don't have enough data.
So like if you look at the stream, right, so it's very biased towards weedplus and rice plus and then we have a handful of wildcats effectively it's just so we're currently actually looking into this kind of using the northern Italian population as a model because it's most easily accessible for us.
Very stable as well, very, very stable. But obviously if we talk about this type of phylogenic analysis, these are always mapping based. So like they are always based on a specific reference genome. And then you basically analyse genetic variation based on this reference genome.
So what we're missing in all of these analysis are actually accessory genomic.
So we can start, you know, getting a little bit of a better picture of genetic diversity independent of the set co-chromosomes.
But we're just starting this so.
And now I forgot the second part of this question.

You know, given that as you get further away from South America, the number of strains that are getting out and spreading around the world is quite limited. What's the risk? You know that that those strains again will actually broaden out the host range and exposed to new environ?

I guess kind of it's a bit of a two-part again. So like first it's a bit of a shame that we're lacking a bit information about the South American population, how it really actually evolved over time.
So the data that we have points towards a shift in the population and most of the isolates that were recently sequenced belonged to this one single image.
But we don't really have good population wide understanding about the diversity that is still present in South America.
So it could be that, you know like one specific image has really taken over.
But yeah, it's very difficult to get data from the affected countries very often.
So there is potential for hybridization.
There's potential for on the transfer, so we in in the lab, we can actually achieve sexual recombination.
And yeah, so we can show that wheat blast has the ability to recombine with endemic isolates.
So we tested this, some immunisolids, but in general it's possible. It rarely happens in nature, but obviously we see that in the wheat blast there’s potential for sexual recombination, it's difficult to determine, you know, how big the potential is.

There's a couple more questions.
So Annie's asked, when you say that the pandemic strains have increased virulence, does this include on the resistant to NS cultivars?

Yeah, the NS worked, relatively good well right after the incursion in 2016.
Especially in combination with other sources of resistance. So like there are currently 2 main sources of resistance against wheat blast.
One is this twin S translocation from ventricular so this is kind of an integration and the second one is called GR.
So like these two seem to work relatively well also against these highly virulent isolates, but I just talked to both people from Bangladesh and people from scimit and they both told me that the twin is translocation is already breaking down. So at the moment it doesn't really (inaudible) genetic resistance. There are a few other resistance genes that have been identified just in the last couple of years.
The problem with some of them at least, is that they are kind of conditionally active depending on the environmental conditions. So often like these tests are done in the lab under control conditions. But in the actual conditions in the field. For example, in Bangladesh or especially kind of regions that are bearing one, some of these resistance genes are useless. SO2NS in combination with this second resistance gene is currently still the best that's on the market.
And the uprising programmes in Bangladesh, start to identifying potential novel sources of resistance, but at the moment it doesn't look so good.

So Lindsay's asked, how is this research commercialised?
And do you sell the intellectual property rights or a patent?

I'm not thinking about the patent at the moment, obviously.
The approach of domain engineering for resistance has been used by many many groups now.
So the concept of this integrated domain is around since 10 years now and probably in the last eight years or seven years we've seen like several examples of the domain engineering popping up.
So I think the potential of, you know, kind of agenting this as a technology is very limited.
It's probably possible to think about IP rights in the context of specific cities.
But yeah, currently again, I'm doing sort of only the fundamental work on this.
So we're not in the stage yet where I would think about IP, so I'm not sure if I would like to - I don't know patent is like, this is probably I have to kind of discuss this obviously with my institutes as well.

Does the Australian wheat industry know what level of resistance is available in our current and upcoming commercial varieties?
We did some work well, but we invested in some work around four years ago.
And because a lot of our genetic material does come out of scimit, through our cage experiments, we have about a third of our current rise, which have got 2NS in it.
So we have some protection, yeah.