Minutes of the AsiaBlight Inter-Regional Online Meeting

September 21, 2022

Attendees:

Name (Alphabetical)	Region	Role
3 unaccounted	Uncertain	Guests
Andrade-Piedra, Jorge	Peru	Coordination Committee and Chair
Ani Widiastuti	Indonesia	Regional Representative
David Cooke	United Kingdom	Guest and Presenter
Guo Mei	Mainland China	Guest
Han Chen	Mainland China	Guest
Jens Grønbech Hansen	Denmark	Guest and Presenter
Jeomsoon Kim	South Korea	Guest
Louise Cooke	United Kingdom	AsiaBlight Advisor and Presenter
Seishi Akino	Japan	Scientific Committee & Regional Representative Member
Md. Rashidul Islam	Bangladesh	Scientific Committee & Regional Representative Member and Presenter
Mukhtabar Tashmatova	Uzbekistan	Regional Representative
Pham Thi Thu Huong	Vietnam	Scientific Committee & Regional Representative Member
Philip Kear	Mainland China	Coordination Committee and Minutes
Saltanat Mambetova	Tajikistan & Kyrgyzstan	Regional Representative
Sanjoy Guha Roy	India	Scientific Committee & Regional Representative Member
Teresita D. Masangcay	Philippines	Regional Representative
Tongle Hu	Mainland China	Scientific Committee Member
Wallace Chen	Taiwan	Guest and Presenter
Waqas Raza	Pakistan	Regional Representative
Xiaoping Lu	Mainland China	Coordination Committee
Xingbin Che	Mainland China	Regional Representative
Xinjie Zhang	Mainland China	Guest
Zhenxin Zhang	Mainland China	Lab specialist for AsiaBlight
Zhijian Zhao	Mainland China	Guest
Zurab Khidesheli	Georgia	Regional Representative

Actions Points

- Jorge suggests that Greg Forbes could be helpful with the proposal development because he is retired with more time on his hands. Jorge suggests that he should follow this up with Jens and David.
- Representatives should complete the survey and request FTA cards if necessary. You can request FTA cards directly through Zhenxin's email: zhenxin.zhang@cgiar.org. The survey is here: https://www.smartsurvey.co.uk/s/YHXJ1A
- Zhenxin will write an email to Dr. Kim regarding South Korea's FTA card requirements
- All presentations should be sent to Philip for him to share with all members.
- Mr. Xingbin Che and Dr. Md Rashidul Islam to discuss exchanges between the two countries.
- Contact Dr. Seishi Akino about presenting at the next AsiaBlight meeting.

Website updates:

- The lab at CCCAP is becoming a hub for Asia for genotyping, which needs to be mentioned on the website. [Also, Guha Roy Lab at Dept. of Botany at West Bengal State University is a hub for Indian isolate sampling and proposed for SAARC countries].
- Upload Louise Cooke's EAPR presentation on the AsiaBlight website
- Philip requested presenters' permission to upload slides onto the AsiaBlight website.
- Protocols for bioagents and chemicals used to control late blight, chemicals to which resistance has developed and regional restrictions.

Presentation 1: Jens Hansen and David Cooke

Global early warning for new and emerging *P. infestans* genotypes:

Step 1. The *P. infestans* global genetic landscape initiative (*Pi*GGL)

- Initiative for a global early warning system for *P. infestans* genotypes, outlining objectives. A funding opportunity.
- What is the objective, and how can a global network help? The network can help improve management with short- and long-term objectives. The short-term objectives of alerts and transboundary warning systems drive management. In the UK, management can be effective if chemical and host resistance work well, but when it fails, a crop can be rapidly lost.
- With the discovery of new genotypes, this information needs to be disseminated early with rapid characterization. But, long-term objectives are also critical; working with breeders is an important aspect. In terms of communicating information about which *P. infestans* lineages are spreading.
- Jens has been developing a BlightTracker APP, for disease surveillance and sampling. It is a goal to receive samples on FTA cards and analyze and get results within a week (one or two days). These samples will be held in a database. Everything will lead to testing and best-practice for sharing information.
- Super goal of being prepared for new and emerging *P. infestans* genotypes. There is a lot of international work being done, but funding is required to do it. Requirements include establishing a global network of labs.
- It would be nice to have would be a more standardized way of phenotyping isolates. Introduction of a method to grow new sources of resistance internationally to get rapid information on failures.
- Comparison between "number of risk days 2020-21." 2021 had a far higher number of risk days than 2020. In 2021, there were 2495 isolates, and over the whole database, there were 42446. They have many clonal genotypes of *P. infestans*. In Europe *P. infestans* reproduces both asexually (clonal genotypes) and sexually (diverse 'other' genotypes), so European *P. infestans* management is challenging. They also have datasets for Africa and Central Asia, South/East Asia, Oceania, and North and Central America. Data from South America within the database are limited, but a lot more data have been generated within Tizon Latino.
- Nine labs will be capable of doing genotyping around the globe. It is important to run the microsatellites (SSRs) in a standardized format.
- David has developed a genotyping method that is used across time to observe genotype development.
- The database used for Euroblight is the same one that is used for Wheat Rust databases, so the maintenance is easier. Global infrastructures for transboundary diseases project idea with David Hughes because funders like BMGF have indicated that they would fund a database that can handle multiple diseases simultaneously.
- Higher resolution genotyping: SSRs have many benefits, but there is interest in knowing more about other areas of the genome outside the areas that SSRs can elucidate. There is interest in mutation rates across effector genes and fungicide resistance genes, for example. Genotyping methods like genome sequencing have potential applications in this area, which may allow bulking

populations together depending on the traits that they have. Sequencing would also allow the user to know about the ploidy and destabilization of the genome over time.

- How could this work? Baseline SSR typing to identify populations/samples to target. The PENSEQ method would use enrichment of target genes followed by Illumina sequencing. Multiplex effector PCR followed by Illumina. Oxford nanopore would allow real-time selective sequencing. There has been a little progress, but not ready to transition yet.
- Standards samples could be passed between groups.
- Ways to get funding beyond the existing system. Currently, providing funding comes from a system where companies may provide funding, but more is required.

Comments & Questions.

- Jorge: CCCAP is becoming a hub for Asia for genotyping so that needs to be mentioned on the website. [Also, Guha Roy Lab at Dept. of Botany at West Bengal State University is a hub for Indian isolate sampling and proposed for SAARC countries].
- Jorge suggests that Greg Forbes could be helpful with the proposal development because he is retired with more time on his hands. Jorge suggests that he should follow this up with Jens and David.
- Rashidul: EU13A2 genotype is predominant in his region and Asia. Do you have plans to compare why the same genotype may have different responses in the host?
- Response from David: Yes, you're right. The original isolates of EU_13_A2 collected in 2005 (published in 2012) have not been compared with genotypes currently circulating in the population. It is not known how the genome of an individual clone varies over time and how that can drive a different phenotype. While the phenotypes of a clone are more similar than they are to other clones, there is still a variation that could be associated with the instability of the genome. It could be due to the silencing of genes. These issues are not related to sexual recombination. This project could answer this question.

Presentation 2: Zhenxin Zhang

FTA Cards Distribution

- At the beginning of the year, a survey was sent to all members. To date, only 7 representatives responded to the survey. [The survey was used to identify each region's requirement for FTA cards].
- Anyone that requires FTA cards should scan the complete the survey and/or email a request directly to Zhenxin Zhang. Zhenxin's email: zhenxin.zhang@cgiar.org. The survey is here: https://www.smartsurvey.co.uk/s/YHXJ1A. Distribution of FTA cards has occurred for 5 of the 7, but he is still awaiting confirmation of the number required by South Korea. Zhenxin will write an email to Dr. Kim regarding South Korea's requirements. Cards have arrived in four regions; one is in transit for Pakistan*. This year, we expect three regions to return their cards having taken samples. But for the Philippines, due to logistical problems, it is very hard to get the FTA cards back. But we will continue working on it.

* Post-meeting note: FTA cards have now reached Dr Raza in Pakistan

Presentation 3: Md Rashidul Islam

Biofungicide and Updates on PLB in Bangladesh

Part 1:

- Bangladesh produces 10 million tonnes of potato annually from 5 million hectares, about 2.6% of world production. 95% of potatoes produced are consumed. Some are used for seed, and some for chips and French fries. Bangladesh also exports its potatoes. With a surplus of 4.5 million tonnes of potatoes. With losses of 290-400 Million USD annually.
- Out of the total requirement for seed potato (0.75 million tonnes): 1% imported seed, 5% private sector, 3% public sector, 24% farmer's improved seed, and 66% is of unknown quality. Using certified seed is a potential solution.
- Bangladesh has five major potato-growing regions, especially in the north and central regions. The major potato varieties are Diamant, Asterix, and Cardinal, among others. Bangladesh is among the top twenty potato-producing countries. Late blight is a significant quality issue for potatoes in Bangladesh because of the three months (Dec-Feb) when low temperatures (10-15C) and high humidity (>90%) occur.
- Bangladesh uses 36,500 tons of pesticides per year. Of that, for potatoes, Bangladesh uses 100 tons per year which may cause a health hazard and affect the environment. In addition, these heavy uses of fungicides can cause a problem of resistance in the pathogen population.
- There is an interest in bio-fungicides in Bangladesh because of the health-related issues associated with fungicide usage. Therefore, fungal and bacterial bio-fungicides are prominent options.
- In 2008, a report was issued by Rahman (Rahman *et al.*, 2008, *International Journal of Sustainable Crop Production* **3**(2), 10-15) indicating that metalaxyl-based fungicides were adequate to control late blight. However, with the emergence of new genotypes of late blight, including EU_13_A2, in Bangladesh, resistance to late blight has become a problem. As a result, 70 isolates from different breeding potatoes are maintained in Dr. Islam's lab. Testing metalaxyl sensitivity on the 70 isolates, 62% (43 of 69) isolates were resistant to metalaxyl, 38% (26 of 69) were intermediate and none was sensitive.
- Due to these "alarming" results, they decided to check the performance of fungicides that farmers could use, including those available on the market. Results, published in a local journal, analyzed fungicides regularly used. All of the tested fungicides wholly inhibited the growth of the EU_13_A2 genotype, explaining why control of late blight has been successful in Bangladesh's last 2-3 years.
- The GEOPOTATO project: Is a warning system that Rashidul believes needs greater dissemination at the farmer level.
- Further, Bangladesh imported two genetically modified (GM) late blight-resistant potato lines developed at Michigan State University for field trials. The Future Fund USA funds the work, but it is uncertain whether they will be used or released for commercialization.

Part 2:

- Assessment of field performance of some formulated bacterial and fungal bioagents. Experiments using bioagents to arrest growth of *P. infestans in vitro* were successful. They observed that the use of the bioagent changed the structure of mycelial growth and stopped the production of sporangia. *Trichoderma paraviridescens* inhibited the growth of *P. infestans* by 46% and *T. erinaceum* by 51.5% compared to the control. Then fungicides were tested for interactivity between bacterial and fungal bioagents. They only found a negative interaction with the chemical fungicides. Therefore in field applications, they first applied the bioagents, and two days later, they applied chemical agents. They observed that same-day applications cause a negative interaction. Growth temperature influences bioagents and different combinations have different effects in field conditions. However, the combined use of bacterial and fungal bioagents along with Curzate M8 reduced late blight severity by 89%, which was higher than using Curzate M8 alone. Experiments were repeated in the growth chamber in 2019-2020. Estimation of the financial return suggests that the use of these bioagent combinations would give an increase in returns. Further experiments were used to schedule appropriate applications of bioagents with chemical agents. For instance, at 53 days after planting (DAP), it is possible to have three sprays of chemical fungicide and a single formulated bioagent. These results indicate that such a combined application of chemical and bioagent together with a sound warning system can reduce fungicide use by more than 50%.

Comments & Questions:

- Saltanat: Did Bangladesh approve GM potatoes for research or consumption?
- Rashidul's response: The country has approved using GM potatoes depending on potentiality. However, we do not know whether they will be successful. He further responded that until now, they had been used for research, but it may be a similar story as BT Brinjal (eggplant). [farmers unofficially adopted BT Brinjal].
- Xingbin: Request to have a copy of Dr. Islam's presentation. He also suggested exchange visits between China and Bangladesh because the situation between the two countries is similar.

Presentation 3: Wallace Chen

Studies of late blight in Taiwan

- The center emphasizes the late blight of tomatoes, but they have also collected some isolates from potatoes. Between 1997-98 there was a severe outbreak of late blight occurred on the potato and tomato. The farmers observed that metalaxyl-based fungicides were not working well, resulting in the quick devastation of farmers' crops. After this incident, the researchers at World Veg wanted to know what had happened, so they took 990 samples. 930 were from tomatoes and 60 from potatoes. This incident caused a significant change in the mindset of Taiwanese researchers. The research concluded that there was a new population in Taiwan, and the new population had quickly displaced the old one. The change took place over a couple of years, but it was a complete change. Until this

point, A2 had not been detected in Taiwan. In Taiwan, the new population was identified as US-11. However, it is unclear where US-11 came from, but some believe it must have been imported on potato tubers. Now, in most cases, the population is wholly displaced. Currently, they cannot find US-1 lineages anymore.

- Because the late blight epidemic was so severe, they wanted to screen the population further to provide help to farmers. So they developed the severity rating scale (0-6). 0 is no symptoms, and 6 is extensive stem damage or death. After developing a rating scale, they tried to understand the virulence structure of isolates to see whether they all express the same virulence or can be separated into different races. So, they composed a set of 6 differential tomato varieties, including different species. Some varieties contain no resistance genes, but others contain PH1, PH2 and PH3, respectively. L3708 and LA1033 are wild species. According to the rating scale, anything over 3.5 is considered susceptible. The race designation is derived from the resistance genes defeated by the isolate.
- They surveyed the isolates that they collected during 2004-2005. They found that they could segregate the races into ten groups. The most aggressive is T1,2,3,4,5. This isolate can affect all the differential hosts and show severe symptoms. The most dominant race in Taiwan is T1,2,3. The race can break down PH1, PH2 and PH3 genes, which are genes found in commercial varieties. In this way, it was observed that there was no effectively resistant variety to the predominant gene. So, much time was needed to identify new sources of resistance from wild species. They also tried to characterize the genotype of the population in Taiwan. They followed the multi-locus system developed by the USDA, using RG57 fingerprinting. Through this endeavor, they found that most populations fall into two lineages: US-1 and US-11. However, they also discovered a new lineage, referred to as TW-1. But they only found one isolate belonging to TW1 and never found it again.
- Before 1998, all collected isolates belonged to US-1, but after 1998 most isolates belonged to US-11. They also tried to develop resistant lines from wild-resistant germplasm, called L3708. They bred to a BC3F3 generation using backcrossing to L3708. In the BC3F6 population, six advanced lines with high resistance were found. After that, they used these materials to create several F1 populations that showed resistance in the field. FMTT795 is used as a commercial variety. They continue to explore different sources of resistance, but because segregation exists, they want to 'purify' the resistance through selfing. Selfed germplasm demonstrated more excellent resistance than parental genotypes. These materials were put into the gene bank. Anyone may request this material if they are interested.
- In addition, they screened for chemical fungicide effectiveness. Firstly, they looked at fungicides frequently used by farmers. They observed that cyazofamid, from Japan, was as effective as Famoxadone+Cymoxanil in controlling tomato late blight in the growth chamber. In addition, they tried using alternative controls using antagonists, neutralized phosphorous salt (NPS) and phytochemical water extract. NPS showed a good result, yielding good fruit under high-disease pressure in Bali, Taiwan. These results demonstrated that NPS could be a suitable alternative because it is not toxic to humans or the environment and costs less than conventional fungicides. For the antagonist, they used *Streptomyces*, and as the Chinese herb, they used Oughon (*Scutellaria baicalensis Georgi*).

Comments & Questions

- Louise: I am interested to know more about the neutralized phosphorous acid. Is that phosphonic acid or phosphorous acid?
- Wallace: That is phosphorous acid.

- Louise: Then, phosphorous acid is a widely used fungicide. It is both directly fungi-toxic and also stimulates the host plant's defenses. It is approved as a fungicide in some countries, but sadly only in a limited way in Europe.
- Wallace: It probably depends on the country. In Taiwan, it is registered as a bio-fungicide that can be used within organic farming systems.
- David: It seems that Taiwan is successful in keeping out other lineages. You have been dealing with US-11 now for decades. What is your secret? Are you still concerned that there could be new populations coming in?
- Wallace: Yes, there are concerns about another population entering Taiwan. However, they consider that US-11 came into Taiwan by planting table potatoes as seed potatoes. They encourage farmers to follow Taiwan's policy that tubers should be verified clean before planting. So, if you use table potato, it may be contaminated with late blight, as well as other fungal or bacterial diseases.

Presentation 4: Louise Cooke

A summary of the EAPR Triennial meeting in Krakow in Poland: 4-8 July 2022.

- The meeting was initially planned for 2020. The first female president was appointed to the EAPR. 200 delegates from 36 countries participated.
- Louise's presentation was entitled: Update on AsiaBlight's efforts to create a course-scale map of *P. infestans* population in Asia. She showed that Regional Representatives cover 14 regions across Asia. Showed examples of selected countries: Bangladesh, India, Pakistan and China, described the progress in genotyping and summarized results.
- Highlights from the meeting were Ian Barker (CIP) gave a keynote speech: Ian highlighted the increasing importance of potatoes in Asia and the role of increasing potatoes because they use much less water than rice. He mentioned that CIP uses potatoes to achieve the SDGs, and 20% of the potato growing area in the global south is occupied by CIP varieties. In addition, high bioavailable iron in potatoes, particularly yellow-fleshed CIP varieties, could cover 50% of iron deficiency in women.
- Highlights from a presentation by Francine Govers from the Netherlands: She worked on *Phytophthora* for many years. She has investigated how it investigates and manipulates its host. They can track zoospores and see them explore their environment. They respond to attractants to locate their host, so blocking the attractant response may be a way for future disease control. They have recently published on how zoospores invade their host. They invade their host using a technique of slicing with sharp, high, full tip, called naifu invasion. This is different from the brute force used by fungi. Once in the host cell, haustoria deliver effectors which manipulate host defenses: these are dynamic, and those recognized by the host can be deleted. If R-gene stacking within potato cultivars is used for sustainable blight resistance, then monitoring the corresponding RXLR effectors in the *P. infestans* populations is essential.
- Francine has collaborators in China that she could introduce if interested.

- Highlights of a poster by Ludwiczewska (Jadwigao Sliwka's group): On detecting genes for resistance to *P. infestans* in selected potato genotypes using PCR markers. They used gene sequencing and designated gene sequencing to analyze the occurrence of resistance genes and their diversity. The project will also analyze the diversity of genes encoding late blight effectors in *P. infestans* populations in Poland and Norway. As part of this work, they also published a review paper on the diversity of R genes in potatoes.
- The last presentation mentioned was from Ingo Hein, who explored novel disease resistance genes.
- The EAPR Triennial meeting will be in Oslo, Norway, 7-12 July 2024, under the Presidency of Arne Hermansen.

Comments & Questions:

- Louise: Please email Louise Cooke if you have any questions about her presentation.
- Md Rashidul: He thinks that CIP should have a special meeting on potato late blight to gather all scientists working on PLB globally.
- Jorge's response: We still have some issues with the pandemic, but it is something that we need to do.

Other items

Preparation for the next meeting

Three candidates from Japan, Georgia and Indonesia.

- Zurab and Ani agreed to present. We need to contact Seishi for confirmation.

Potential date of next meeting.

- December 15 2022

Thanks for the excellent work

Louise says thank you to Saltanat for the very nice report on her activities collecting samples. In addition, Louise requests that people send correct details of the sources and exact geographical locations, the potato cultivars, et cetera.

Miscellaneous

- Louise suggests putting the EAPR meeting slides on the AsiaBlight website

- Jorge suggests putting all the presentations on the website.
- Rashidul expects the FTA cards sent to Bangladesh to arrive soon.
- Saltanat requests support for funding that would allow the return of the cards.
- Pham requested protocols for using biofungicide and chemicals to control *P. infestans*. And which genotypes are resistant to which chemicals and which chemicals are forbidden by the EU.
- Jorge should be able to do that. But also suggests that this information is also available on the EuroBlight website.
- Louise responds to Pham's request: The only confirmed resistance to chemicals in Europe [potentially worldwide] is metalaxyl and fluazinam
- David: Suggestion of shorter updates to allow more countries to contribute.

Post-meeting notes:

- Information on the effectiveness of late blight fungicides is available on the EuroBlight website at https://agro.au.dk/forskning/internationale-platforme/euroblight/control-strategies/late-blight-fungicide-table. If you click on the 'Only EU' option at the top of the Table, those fungicides not now permitted to be used in the EU are excluded from the Table.
- The Table does not provide detailed information on the occurrence of resistance to phenylamides (e.g. metalaxyl) and fluazinam. Briefly, resistance to phenylamide fungicides is present in numerous genotypes of *P. infestans*, notably EU_13_A2, which is almost invariably phenylamide-resistant. Resistance to fluazinam has only been associated with genotypes EU_33_A2 and EU_37_A2, which do not seem to compete successfully with other genotypes in the absence of fluazinam usage. As far as we are aware, EU_33_A2 and EU_37_A2 are only found in Europe and have not been identified in Asia.

Want to watch the meeting again?

Meeting video link:

https://cgiar-my.sharepoint.com/:v:/g/personal/p_kear_cgiar_org/EfEitSNWCwJEsJj3D1cHzgkBs_vEDUr0EYt42AfSvBgWZQ?e=jXvDAH

Password: AsiaBlight2022

Expiration date: October 14, 2022.