

Recent developments: late blight in Asia

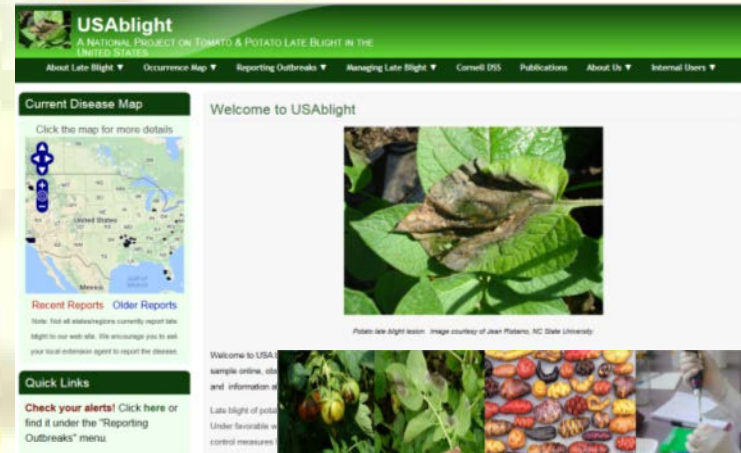
AsiaBlight

Louise R Cooke,
Ruofang Zhang, Lei Wu, Si Hui Chen,
Greg Forbes



Success of EuroBlight network inspired :

- USABlight
- Tizón Latino
- AsiaBlight
- *AfricaBlight?*



Origins of AsiaBlight:

Nepal, November 2014

Workshop of experts from 10 Asian nations approved creation of AsiaBlight. Proposed 4 thematic components plus 1 management component (co-ordination, communications, monitoring, evaluation).

China, July 2015 (at World Potato Conference)

First meeting of AsiaBlight: discussed coarse-scale map as an initial activity.

India, September 2015

Indian scientists at 3rd International *Phytophthora* Symposium agreed that co-operating on pathogen population change within AsiaBlight would be beneficial.

M&E

Fungicide efficacy

Host plant resistance

Disease management &
forecasting

Pathogen diversity and
evolution

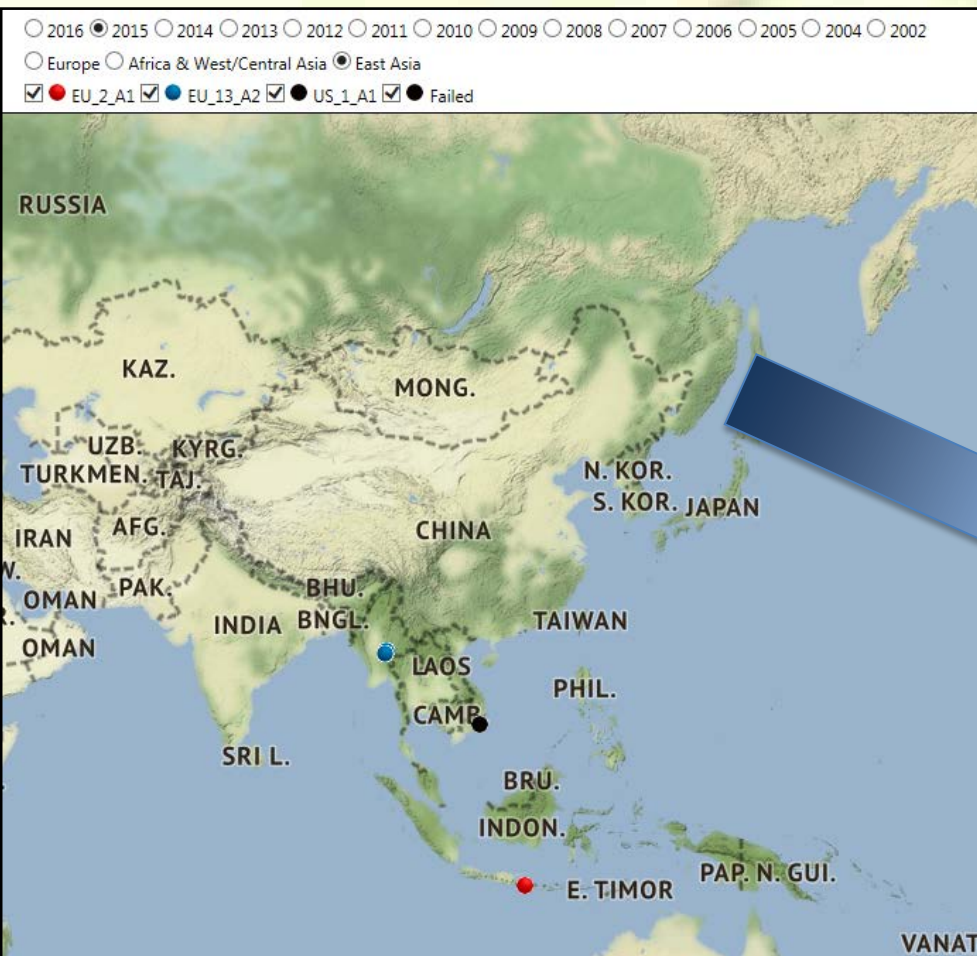
AsiaBlight: initial objectives

- Map the current Asian *Phytophthora infestans* population
- Demonstrate the potential of Public-Private Partnerships initially between public sector research institutes and agrochemical companies
- Develop team spirit among Asian partners - promote collaboration for future activities

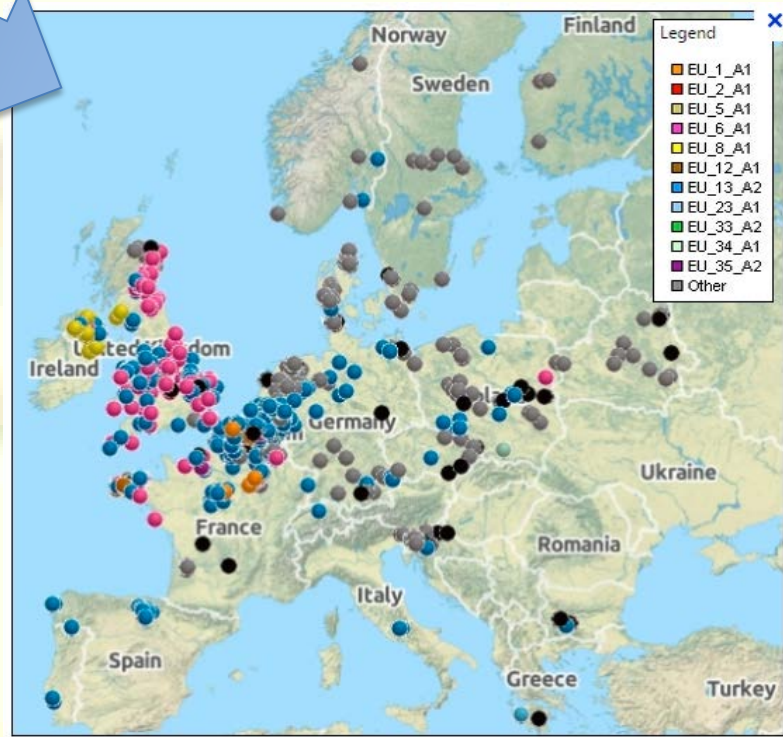


AsiaBlight: map genotypes

From this....



... to this



How has it been achieved so far?

- FTA cards for most countries funded by Bayer; some sampling is occurring from ongoing projects (China, Korea)
- Distribution of FTA cards and genotyping offered by Dr Ruofang Zhang (and her team!)
Inner Mongolia Potato E & T Research Center, Inner Mongolia University, Hohhot, China
- Overall supervision volunteered by Louise Cooke

AsiaBlight: proposed timeline

	2016				2017				2018	
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
Task 1	Planning									
Task 2			FTA Purchase, distribution							
Task 3				FTA collection						
Task 4							FTA analysis, mapping			
Task 5									Publication	

Bangladesh
China*
Georgia
India
Indonesia
Japan
Korea*
Nepal
Taiwan
Tajikistan
Uzbekistan
Vietnam
Armenia
Bhutan
Kazakhstan
Kyrgyzstan
Malaysia
Myanmar
Pakistan
Thailand
Philippines

Initial AsiaBlight project: a coarse-scale map of the current late blight population in Asia

Country scope



Sampled for AsiaBlight

* Sampled and genotyped by other projects


Not sampled for AsiaBlight

AsiaBlight: problems

- Asia is a large, very geographically dispersed and politically disparate region
- Co-operative links between countries are limited
- Countries differ greatly in their organisational structures, political attitudes to regional co-operation, plant health legislation and so in their ability to participate
- Has not been possible to get samples from all countries; thus partial map will be developed

How is it going?

AsiaBlight initial timeline

	2016				2017				2018	
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
Task 1										
Task 2										
Task 3										
Task 4										
Task 5										

- January-June 2016: identification of country contacts, and funding for FTA cards (Bayer Regions APAC 1 and APAC 2)

How is it going?

AsiaBlight initial timeline

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Task 1										
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- FTA cards purchased July 2016
- Dr Lei Wu: July 2016 sent out cards, sampling instructions, forms for data recording (number of cards per country based on potato area, sampling ability, generally 10-25 cards/country with 4 sampling areas/card)

How is it going?

AsiaBlight initial timeline

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Task 3							
Task 4							
Task 5							

AsiaBlight
Protocol for sampling *Phytophthora infestans* DNA using FTA cards 2016

The process takes longer to explain than to do - it's really simple!

- Use 1 card with 4 sampling areas (circles) per site (field).
- Sample 4 lesions per infected site. If only one plant is infected, collect 4 separate lesions from the same plant. If there are numerous infected plants, collect 1 lesion from each of 4 separate plants distributed across the site.
- Correct sampling is critical. Select leaflets with single, fresh, nicely sporulating lesions for each sample (Figure 1). Avoid dead leaves, old or dry lesions, leaflets with many lesions, wet, water soaked (bacterially infected) looking leaves and those with small restricted lesions.
- Label the FTA card with a reference number (if not already done). Provide the rest of the information on the sample form.
- Cut out a piece of sporulating lesion approx. 1.2 cm square at the edge of the lesion (Figure 2) using sterilised scissors or scalpel (use an alcohol wipe).
- Place the lesion sample on one clean circular sampling area on the FTA card sporulating side down. Do not touch the FTA card sampling area except with the lesion sample!
- Replace the FTA card's cover sheet over the lesion sample and apply moderate rubbing pressure to the sample through the cover sheet using a blunt object (e.g. the blunt end of a marker pen or pestle). Don't rub too hard or you will damage card. When green leaf extract is visible on the back of the FTA card, the collection process is complete.
- Remove excess plant residue from the FTA card, ensuring that no large pieces of plant tissue remain stuck to the card (Figure 3). It doesn't matter if some small bits remain.
- Air-dry the FTA cards for at least 1 hour at room temperature.
- Store dry FTA cards separately in paper envelopes.








Figure 3. FTA card with samples after they have been used for SSR analysis (DNA has been extracted from the 2 mm disks punched out of each sample) and clean-up of card with lesion debris.

AsiaBlight 2016 *Phytophthora infestans* SAMPLING FORM

Please write clearly!

COMPULSORY:

FTA Card Reference number

Sampler name

e-mail address

Country -

Nearest Town -

Postal Code/Zip Code

Q1 gpc coordinates (decimal)

Q2 gpc coordinates (Deg, min, sec)

Source: Production field/Year

Sampling date

Host (Potato or Tomato)

Cultivar

Remarks

Disease level at sampling High Medium Low

OPTIONAL

Product(s) applied (if known)	Application dates or number of applications (if known)
<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>
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- Sampling/FTA collection started August 2016, completed for some countries, ongoing or further sampling planned in 2017, will be completed by end 2017
- Cards sent back to Dr Lei Wu

How is it going?

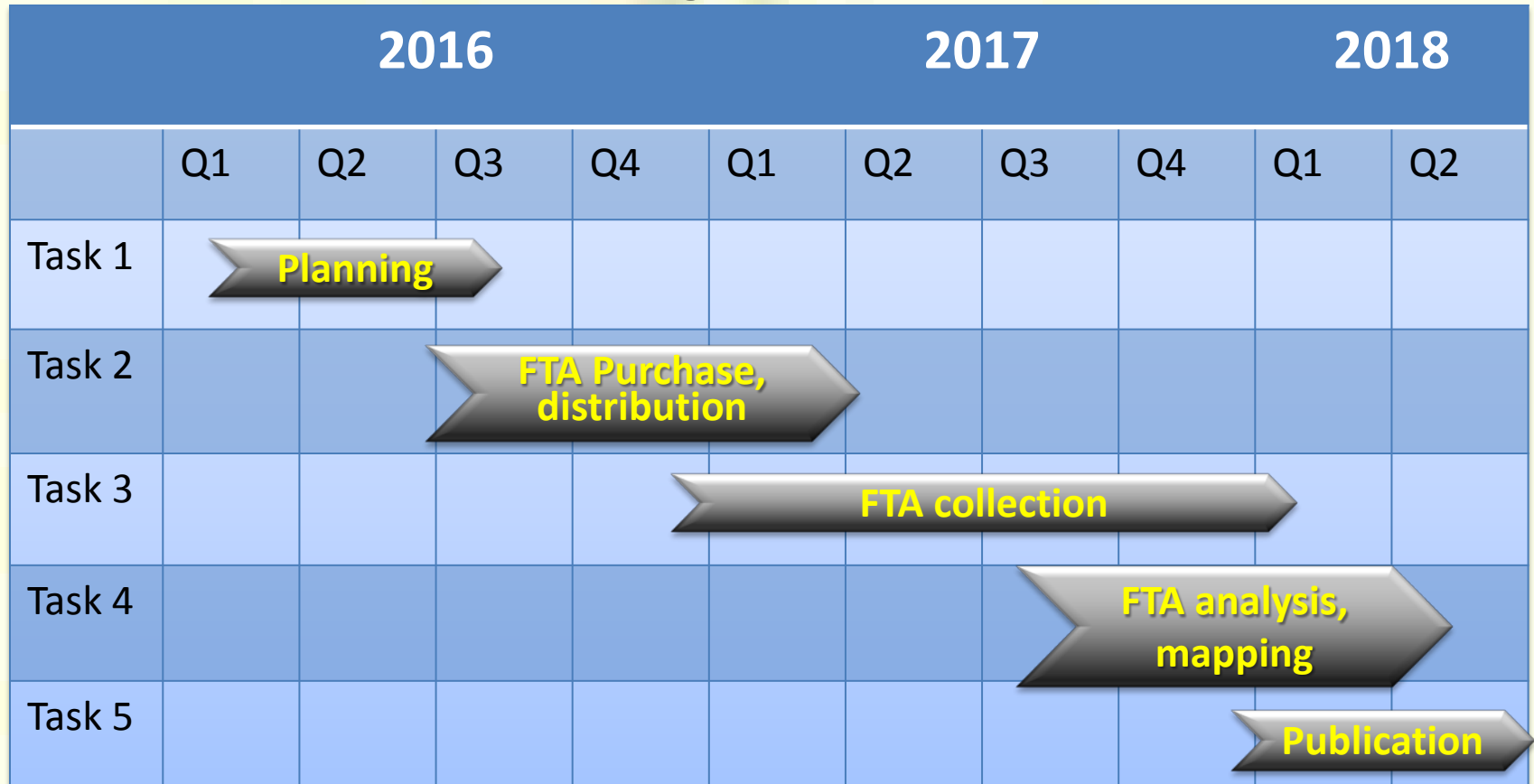
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- Sihui (Master's student): started the SSR analyses December 2016, now joined by Lan Yu (PhD student), they are working on the SSR analyses and genotyping together helped by Jonathan Yuen and by David Cooke (who supplied DNA genotype standards) and are advising on interpretation
- Should be completed by early 2018

How is it going?

AsiaBlight initial timeline



- Planning to write-up for publication in 2018

More detail on progress

Country	No. of FTA cards	Sampling status	Sampling details
Georgia Tajikistan Uzbekistan	10 10 10	Georgia: sampling 2016, further sampling 2017 Tajikistan: hoping to sample 2017 Uzbekistan: no current contact	Georgia: 10 potato crops sampled
India	100	No AsiaBlight sampling to date	
Nepal	25	Sampling in progress	Potato crops sampled in low, mid zones; high hills yet to be sampled
Bangladesh	25	Sampling complete	25 potato crops sampled
Indonesia	10	Sampling complete	10 potato crops sampled, 6 locations
Vietnam	10	Sampling in progress	4 potato crops sampled, 6 tomato crops in highlands to be sampled
Taiwan	10	Sampling complete	10 tomato crops sampled
Japan	10	Sampling complete	10 potato crops sampled

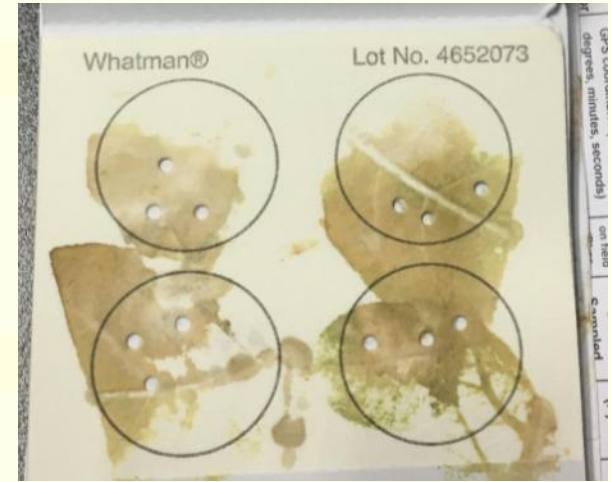
DNA extraction

Country	No. of FTA card samples with DNA successfully amplified
Georgia	10
Bangladesh	25
Indonesia	10
Vietnam	4
Taiwan	20
Japan	10
Total	79

SSR analyses completed on all samples except those from Vietnam

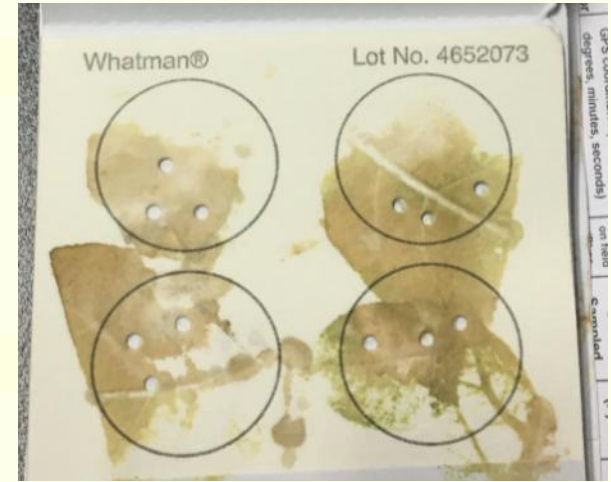
Preliminary results:

- Some missing data due to poor quality DNA (old lesions)



Preliminary results:

- Some missing data due to poor quality DNA (old lesions)
- Standardisation of allele sizing in progress to allow comparison with EuroBlight database and hence...
- Identification of genotypes
- Blue 13 appears widespread



AsiaBlight: FTA card sampling and genotyping

- In addition to the FTA cards sent out from Hohhot, cards have also been distributed and/or samples collected by EuroBlight colleagues involved in other projects in Asia
- Geert Kessel, Huub Schepers & colleagues: sampling in Bangladesh, India, Indonesia, Myanmar, South Korea, Vietnam
- Chris Ursell: sampling in Java
- Catherine Chatot: sampling in Sri Lanka
- David Cooke: distributed FTA cards in Vietnam
- Probably others have done this too!

Other achievements:

- Initial PPP successful
- Developing sense of collaboration in Asia
- AsiaBlight brand recognition
- Success in getting SSR 12-plex implemented in China

Next steps for activity:

- **Complete sampling**

- Nepal, Vietnam ongoing
- Pakistan, Philippines, Tajikistan: hoping to sample 2017
- Georgia, Indonesia: further sampling in 2017
- Kyrgyzstan: possible contact identified

We are exploring ways of including India

We lack current contacts for sampling in:

- Armenia
- Bhutan
- Malaysia
- Myanmar

Can anyone help?

Next steps for activity:

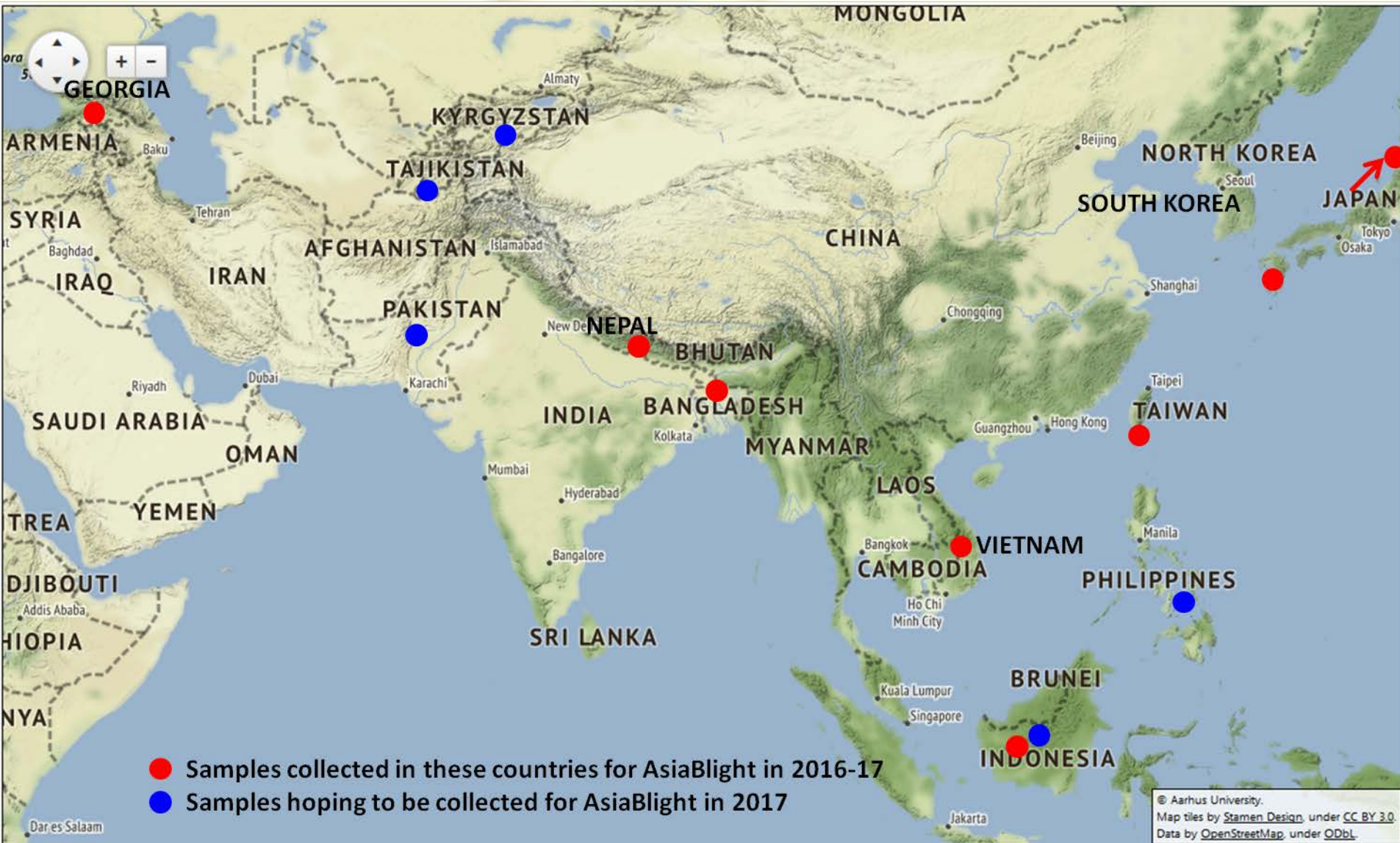
- **Complete genotyping**

Need to repeat some samples because of missing data (poor DNA quality),
assign genotypes (standardise allele sizing),
analyse additional samples, aiming for completion by end 2017.

- **Mapping and publication**

Adding analysed data to EuroBlight map straightforward, but requires sample locations and agreement of contacts.
Planning to write-up in 2018.

Next steps for activity: mapping



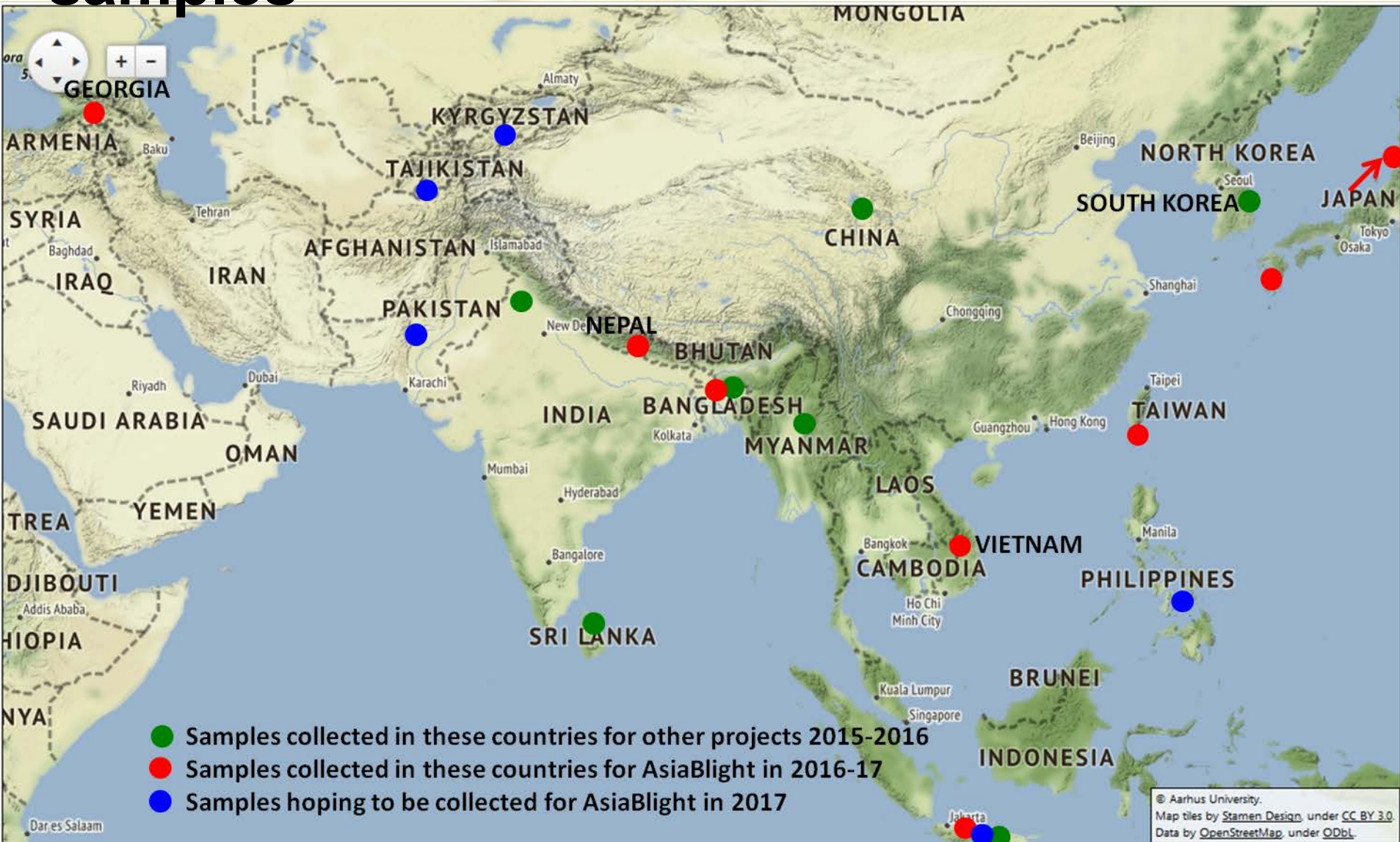
Samples collected by others 2015



Samples collected by others 2016



Next steps for activity: map integrating all samples



Next steps for AsiaBlight:

- **Proposal development**
 - Build on current experience
 - Involve more private sector partners
 - Include one or more new thematic areas (LB management, host resistance)
- **Satellite meeting at relevant conference**
- **Greater Web visibility (via EuroBlight and/or CIP?)**

Ideas for projects in future

- **With continued minor funding**
 - A second mapping exercise which focuses on tomato
- **With more significant funding**
 - Blight Learning & Innovation Centers with field trials of fungicide efficacy, host resistance including DSS and pathogen studies?
 - Use of CIP or other simple DSS?
 - Fungicide sensitivity?

AsiaBlight: Acknowledgements

- Bayer (Regions APAC 1 & APAC 2)
- David Cooke
- Jonathan Yuen
- Country contacts
 - Bangladesh: Abdullah-Al-Mahmud, Monower Hossain, Ebna Habib Md. Shofiur Rahaman
 - CACs: Zurab Khidesheli, Rusudan Mdivani and colleagues
 - Indonesia: Ineu Sulastrini, Koko Tjintokohadi
 - Japan: Seishi Akino
 - Nepal: Buddhi Sharma
 - Taiwan: Rishi Burlakoti, Wallace Chen
 - Vietnam: Rene van Rensen, Ho Ngoc Anh

AsiaBlight

- We welcome your ideas, offers of collaboration, funding.....
- Thank you for your attention!