

Recent developments: late blight in Asia - AsiaBlight

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SUMMARY

The formation of a late blight network for Asia, AsiaBlight, was first proposed in 2014. In 2015, it was agreed that the initial activity should be generation of a coarse-scale map of the *Phytophthora infestans* population in Asia. This has been progressing in 2016 and 2017 with the assistance of the Inner Mongolia Potato E & T Center, Hohhot. FTA cards (funded by Bayer) have been distributed from Hohhot to contacts in ten Asian countries who have collected late blight samples and returned them to Hohhot for genotyping. In addition to FTA cards sent out under the auspices of AsiaBlight, late blight samples have also been collected on FTA cards elsewhere in Asia by other researchers. Although AsiaBlight is a project with minimal resources, it has achieved growing recognition, a degree of regional collaboration and limited but successful private-public partnership. The challenges in co-ordinating a late blight network without a dedicated budget across a large geographic region with limited co-operative links and many different potato and tomato regions are discussed and possible future activities considered.

KEYWORDS

Phytophthora infestans, China, SSR, population structure

INTRODUCTION

The success of EuroBlight has inspired the creation of other international late blight networks, notably USABlight and Tizón Latino. Although in recent years there have been a number of publications on the late blight population in Asian countries, relatively few of these reported nationwide studies and very few have used markers allowing comparison with populations in other parts of the world (Forbes, 2015). The aggressive genotype 13_A2 (Blue 13) has been detected in a number of Asian countries including China (Li et al., 2013b) and India (Chowdappa et al., 2015), but the extent of its spread is unknown and the implications of its presence are often not taken into account in late blight management. At a meeting in Nepal in late 2014, organised by the International Potato Center (CIP) with the National Potato Program of Nepal,

25 researchers representing 12 Asian countries agreed that there was a need for coordination of late blight research in Asia. A roadmap to create a proposal for a region-wide network of collaboration on potato late blight was developed and named AsiaBlight. This was further considered at a meeting in China in July 2015 where participants were strongly in favour of such an approach and the generation of a coarse-scale map of the *P. infestans* population in Asia was proposed as an initial activity. This would serve as a baseline for pathogen studies and underpin future endeavours of AsiaBlight to improve on-farm disease management. In September 2015, Indian scientists attending the 3rd International *Phytophthora* Symposium also agreed that co-operation on pathogen population change within AsiaBlight was needed. To progress this, in the absence of any specific funding or staff, in late 2015 Louise Cooke (recently retired from the Agri-Food & Biosciences Institute, Belfast) was approached to act as a voluntary coordinator for the initial AsiaBlight mapping project and started this role in January 2016. This paper reports progress since then.

PROGRESS TO DATE

The initial primary objective of AsiaBlight was to generate a coarse-scale map of the *P. infestans* population in Asia, but additional associated objectives were to demonstrate the potential of Public-Private Partnerships (initially between public sector research institutes and agrochemical companies) and to develop a team spirit among Asian partners in order to promote collaboration for future activities.

ORGANISATION OF SAMPLE COLLECTION

It was agreed that the collection of late blight samples should follow the EuroBlight model, with contacts in Asian countries being asked to collect *P. infestans* DNA from late blight lesions onto FTA cards (Whatman Classic FTA cards, with 4 sampling areas per card, 10-100 cards per country depending on potato production area). A time-line was proposed (Figure 1) which has served as a yardstick for measuring progress and demonstrates that the project is progressing more or less on as projected, albeit with a number of challenges.

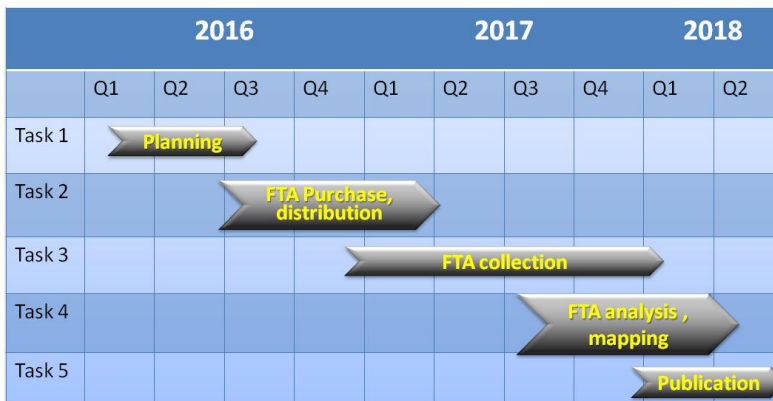


Figure 1. Time-line for AsiaBlight initial project (a coarse-scale map of *Phytophthora infestans* in Asia) as proposed in January 2016

The first challenge was to fund and source FTA cards and determine how they should be distributed. This was organised with the assistance of the CIP Office, Beijing. Bayer (Regions APAC 1 and APAC 2) agreed to fund the purchase of 500 FTA cards (obtained from a supplier in China) and Ruofang Zhang volunteered the assistance of her laboratory and staff in the Inner Mongolia Potato E & T Research Center to distribute the cards and to genotype the resultant *P. infestans* DNA samples.

Instructions for sample collection (based on the protocol developed by EuroBlight) and standard forms for detailing sample information (including site location, host, cultivar and disease level at sampling) were prepared. Contacts were requested to use one FTA card per site and to sample four separate actively sporulating lesions from each site where possible.

COUNTRY CONTACTS AND SAMPLING

Contacts were identified with the assistance of CIP scientists and other researchers. The late blight populations in China and the Republic of Korea were already being investigated in ongoing projects, so these countries were excluded from those to which FTA cards would be sent.

In July 2016, FTA cards, sampling instructions and sample forms were sent from Hohhot to contacts identified and willing to participate in Bangladesh, Georgia, India, Indonesia, Japan, Nepal, Taiwan, Tajikistan, Uzbekistan and Vietnam. Armenia had planned to take part, but the contact there had to withdraw because of changed responsibilities. After the cards had been sent out, it was found that samples could not be collected from Tajikistan and Uzbekistan owing to re-organisations and from India because of biosecurity legislation prohibiting pathogen DNA from being sent out of the country; this resulted in the loss of these cards. During May 2017, additional cards were sent from Hohhot to contacts in Georgia, Indonesia, Pakistan and the Philippines and in July 2017 to Tajikistan (contacts in Indonesia, Pakistan and the Philippines were Bayer personnel).

Initial attempts failed to find contacts able to participate in a number of countries including Armenia, Kazakhstan, Kyrgyzstan, Malaysia and Thailand.

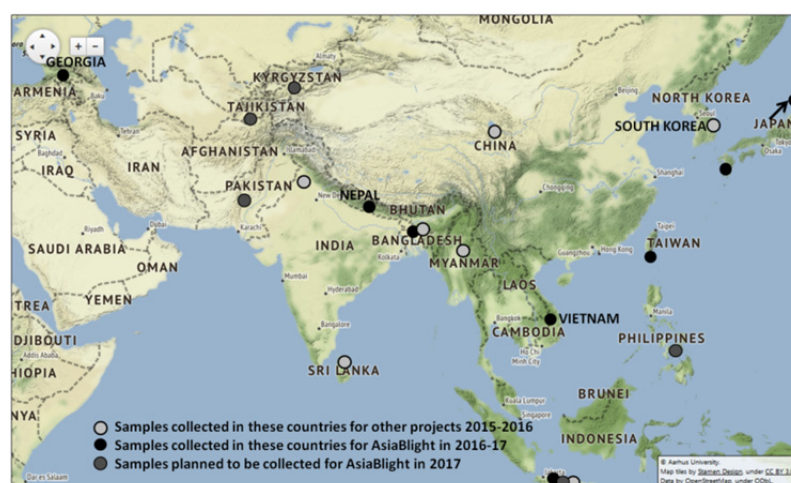
In addition to the FTA cards sent out under the auspices of AsiaBlight, late blight samples were also being collected on FTA cards elsewhere in Asia by other researchers, who submitted them to the James Hutton Institute (JHI) for genotyping and mapping. These included Geert Kessel, Huub Schepers & colleagues from Wageningen University & Research (samples from Bangladesh, India, Indonesia, Myanmar, South Korea, Vietnam), Chris Ursell (samples from Java), Catherine Chatot (samples from Sri Lanka) and David Cooke (samples from Vietnam).

SAMPLE SUBMISSION

FTA cards with sampled *P. infestans* DNA were returned by the sample collectors to Hohhot along with completed sampling forms. Details of sampling and FTA cards returned to Hohhot date (October 2017) are shown in Table 1. Figure 2 shows the locations from which these samples and those collected by other researchers were obtained.

Table 1. *AsiaBlight FTA card sampling of Phytophthora infestans to October 2017*

Country	No. of FTA cards sent and when	Sampling status	Return of FTA cards to Hohhot
Bangladesh	25 (July 2016)	Samples collected from 25 potato crops, January 2017	Cards returned February 2017
Georgia	10 (July 2016) 10 (May 2017)	Samples collected from 10 potato crops, August 2016. Further sampling planned, summer 2017.	2016 cards returned September 2016. 2017 cards not yet returned.
Indonesia	10 (July 2016) 20 (May 2017)	Samples collected from 10 potato crops, October 2016. Further sampling planned summer 2017.	2016 cards returned December 2016. 2017 cards not yet returned.
Japan	10 (July 2016)	Isolates obtained from 10 potato crops (May and August 2016), isolates sampled onto FTA cards	Cards returned December 2016.
Nepal	25 (July 2016), 9 additional cards located locally.	Samples collected from 24 potato crops (November-December 2016, May-June 2017) and 10 tomato crops (February, June 2017).	Cards (34) returned July 2017.
Taiwan	10 (July 2016)	Isolates obtained from tomato crops (December 2014 to August 2016), sampled onto FTA cards.	Cards returned February 2017.
Vietnam	10 (July 2016)	Samples collected from 4 potato crops, February 2017, more samples to be collected later.	Cards (4) returned February 2017.
Pakistan	20 (May 2017)	Cards received.	Not yet returned.
The Philippines	10 (May 2017)	Cards received.	Not yet returned.
Tajikistan	10 (July 2017)	Cards received.	Not yet returned.

**Figure 2.** *Late blight samples collected in Asia from 2015-2017.*

DNA EXTRACTION AND GENOTYPING

DNA has been extracted from the FTA card samples from Bangladesh, Georgia, Indonesia, Japan, Taiwan and Vietnam and analysed using 12-plex SSR (Li et al., 2013a) with DNA from standard genotypes (provided by D.E.L. Cooke, JHI) included for reference. Some data are missing because of poor quality DNA where the late blight lesions were rather old with insufficient active sporulation. Standardisation of the allele sizing to allow identification of genotypes is in progress, but has proved challenging. A need for support in sizing and naming alleles has been identified and will be the subject of an online workshop by D.E.L. Cooke and possibly also a laboratory visit. For this reason it has not yet been possible to inform country contacts of the *P. infestans* genotypes identified in their samples.

CHALLENGES AND SUCCESSES

A number of challenges have been associated with the development of AsiaBlight:

- Asia is a large, very geographically dispersed and politically disparate region, with many different potato and tomato seasons.
- Co-operative links between Asian countries are limited.
- Countries differ greatly in their organisational structures, political attitudes to regional co-operation, plant health legislation and thus in their ability to participate.
- It has not been possible to identify contacts and get samples from all Asian countries that grow potatoes (or tomatoes) so a partial map will be developed.
- AsiaBlight relies on staff in Hohhot to send out and receive back FTA cards and on the goodwill and cooperation of local researchers to collect samples and return cards at their own expense; transferring cards within the region has often proved difficult and slow.
- In a number of cases cards have not yielded good quality *P. infestans* DNA because the need to sample active lesions has not been fully understood.
- The 12-plex SSR is a complex technique to implement particularly when following published protocols rather than learning hands-on. The two postgraduates who have worked on this in Hohhot have done an excellent job in getting the technique running there, but have moved on. There is a need for continuity and for support in genotype identification to generate publishable results by 2018 (as originally planned).
- AsiaBlight does not have its own funding and is benefitting from voluntary assistance, but providing continuity of funding and personnel is going to be important for the future.

Despite these challenges, there has been substantial progress over the past two years. AsiaBlight is becoming known as the late blight network for Asia and has demonstrated the value of Public-Private Partnerships. Other researchers working on late blight projects in Asia have proved keen to participate and have submitted data for inclusion in the mapping hosted by EuroBlight. Examination of preliminary SSR results from FTA cards sent out in 2016 (by D.E.L. Cooke) has indicated that the *P. infestans* genotype 13_A2 (Blue 13) is widespread in Asia. AsiaBlight has also acted as a stimulus for proceeding with an online virtual Workshop on allelizing to be given by David Cooke, which will include not only participants in Europe and China, but also Africa and South America.

NEXT STEPS

The 2017 sample collection is to be completed and all samples collected to date need to be genotyped (requiring some samples to be repeated because of missing data and the need for

standardization of allele sizing and genotype assignment). Only after the genotype identifications have been confirmed in the presence of standard genotype samples will it be appropriate to inform all those who submitted samples of results. It is planned to give AsiaBlight a web presence within the EuroBlight website, however, the co-operators agreement will be sought before genotype data are uploaded to the EuroBlight map. Cooperators will also be involved when results are written up for publication (it is proposed that all who submitted samples should be included as authors on any publication, subject to their agreement), which it was originally hoped would be achieved in 2018.

Several activities have been discussed to enhance AsiaBlight in the future, including i) finding a way to include researchers from India and other important potato-producing countries not sampled, involving more private sector partners, developing a second map with a focus on tomato and holding an AsiaBlight Workshop, the latter most likely to be hosted by China. Should additional significant funding be secured, other research areas are envisaged, such as the development of 'Blight Learning & Innovation Centers' (with field trials of fungicide efficacy, host resistance, pathogen studies and DSS) or the study of pathogen mutations or genotypes associated with reduced fungicide sensitivity.

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