Eleventh EuroBlight workshop
Hamar (Norway), 2008

Characteristics of *Phytophthora infestans* isolates from China

YING LI[1], THEO VAN DER LEE[2*], JIEHUA ZHU[3], GUANGHUI JIN[4], CHENGZHONG LAN[5], BOWEI LIU[6], RUOFANG ZHANG7, ZHIJIAN ZHAO[8], YANLI YANG[9], SANWEN HUANG[1*], EVERT JACOBSEN[2]

[1]Institute of Vegetables and Flowers, China
[2]Wageningen UR, the Netherlands
[3]Hebei Agricultural University, China
[4]Heilongjiang August First Land Reclamation University, China
[5]Fujian Academy of Agricultural Sciences, China
[6]Sichuan Academy of Agricultural Sciences, China
[7]Inner Mongolia University, China
[8]Yunnan Agricultural Academy, China
[9]Yunnan Agricultural University, China;

*Correspondence:
Theo van der Lee. E-mail: Theo.vanderlee@wur.nl , Wageningen UR, The Netherlands

SUMMARY
In several of the main potato production area's in China the climate is favorable for *P. infestans* and consequently, potato late blight is one of the most serious threats to potato production in China. As part of a comprehensive monitoring program that we could establish for China we started genotypic studies on the current *P.infestans* population in China. We found much more genetic variation than previously reported. Nevertheless, the three mitochondrial haplotypes (Ia, IIa, IIb) and the SSR genotypes found in Chinese isolates were all strongly related to the region of origin. Many genotypes, as determined by multilocus SSR markers, are unique to specific regions and only two genotypes were found in multiple provinces located in the North of China. The genotyping analysis shows strong restriction of the spread of *P. infestans* but this could change radically if infected seed potatoes are transported between the different regions in China. Therefore we are currently intensifying our sampling and aim to set up a monitoring program similar to Euroblight.

KEYWORDS
Phytophthora infestans, late blight, population diversity, clonal lineage, China

INTRODUCTION
The late blight caused by *Phytophthora infestans* is one of the most devastating diseases of potato. In many potato growing area's the climate in China is favorable for *P. infestans* and, as a result, potato late blight is one of the most serious threats for potato production in China. The A2 type was firstly detected in northern China in 1996 (Zhang *et al*., 1996), and has since spread as far south as Yunnan (Zhao and Zhang, 1999), near the border with Vietnam. Both the A1 and A2 pathogen exist in China, although as yet there is no evidence that they have recombined into a more adapted or more aggressive variant of the pathogen (Yang, Zhu, and Zhang, 2008).

PPO-Special Report no. 13 (2009), 197 - 202

The potato agribusiness is booming in China in recent years (Chen and Qu, 2008; Qu *et al*., 2005). The national-wide commercial transportation and other trade activities are expected to increase in coming years consequently; the migration of *P. infestans* in China may be national-wide as well. Understanding the current situation of the *P. infestans* population in China, as well as monitoring shift and trends in the future is important for rational short- and long-term resistance management and potato breeding. Unfortunately, the current population diversity of *P.infestans* in China is not well known. In recent years Late blight epidemics in China turned to be more difficult to forecast and to manage possibly because of changes in the *P. infestans* population. Management strategies and forecasting systems depend on an understanding of its population genetics for instance the occurence of a sexual cycle. It is also important to know which virulence factors are present in the regional pathogen populations, because this will identify which potato varieties will be susceptible to the prevailing *P. infestans* population in a particular region. The most recent study on *P. infestans* isolates collected between 1997 and 2003 from Northern China showed that all these isolates belonged to the same clonal lineage (Guo *et al*., 2009). However, virulence identification indicated highly diverse within this clonal group.

The objective of this study was to explore the level of genetic diversity within the modern *P. infestans* population in China using both classical approaches and highly-informative molecular markers (Table 1). A comprehensive survey of *P. infestans* isolates in whole landscape of China was performed. *P. infestans* isolates from six potato regions were mainly sampled in 2006 and 2007. Finally, 118 isolates were obtained providing an adequate view on the *P. infestans* population in order to understand the current diversity and trends of the *P. infestans* population in China.

MATERIALS AND METHODS

Isolate sampling

Potato leaves with a single lesion were collected from five regions in China (Figure 1), placed the leaves individually in plastic bags or 9cm Petri plates containing 1.5 % water agar, and incubated the leaves until sporulation at 15°C at a light intensity of 12 Wm^{-2} and 16 hours. Infected leaves were sectioned into 0.5 cm^2 pieces, placed under tuber slices. After 5-7 days at 20°C, hyphe were transferred to pea agar (Goodwin, Drenth, and Fry, 1992; Shattock *et al*., 1990). In total, 118 isolates were obtained and cultured (Table 1).

Table 1. Origins and characteristics of *Phytophthora infestans* isolates collected in China

Region	Year	Isolates	Mating type	MtDNA	SSR
Northeast	2004-2006	29	18	17	17
Inner Mongolia	2006	25	14	14	14
Hebei	2006-2007	15	4	14	14
Sichuan	2007	18	15	16	18
Yunnan	2004-2006	15	8	10	10
Fujian	2007	16	10	14	15
Total		118	69	85	88

DNA extraction

Isolates were grown in pea broth at 20°C for 5 days and after 5 days the mycelia were collected into 8-strip tubes (Genomic DNA was isolated from 20 mg of lyophilized mycelium using the PUREGENE DNA isolation kit (Gentra, Minneapolis, MN) following the manufacturer's instructions and eluted with 50 µλ ultra-pure water. DNA extracts were stored at -20 °C.

Haplotype test

Mitochondrial haplotypes were determined using the PCR-RFLP method of Griffith & Shaw (Griffith and Shaw, 1998). Restrict digestions of amplified regions P2 (MspI) and P4 (EcoRI) allowed differentiation of four mitochondrial (mtDNA) haplotypes Ia, Ib, IIa and IIb.

Microsatellite analysis

Eight microsatellite markers were selected for microsatellite analysis of all isolates (using fluorescently labeled primers, from PRI). The forward primers (of 8 primer pairs) were labeled with either FAM or HEX. Amplification reactions consisted of 10 ng template DNA, 200 µM dNTPs, 0.8 U *Taq* DNA polymerase (Roche, Indianapolis, IL), 1.5 mM $MgCl_2$, and different volume of each primer in a 20 µl reaction volume. Amplifications were run in a PTC200 thermocycler (MJ Research, Waltham, Massachusetts, USA), with initial denaturation at 94°C for 2 min, followed by 13 touch-down cycles of 94°C for 30 seconds, from 66 to 53°C for 30 seconds, and 72°C for 30 seconds, then by 28 cycles of 94°C for 30 seconds, 53°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 7 min. 1-2 µl of PCR product from successful amplifications were added to 1 µl de-ionized formamide loading buffer, denatured at 92°C for 3 min. The resulting amplification products were sized by capillary electrophoresis on an automated ABI 3730 using the molecular standard GeneScan-500 ROX and GeneMapper4.0 software (Applied Biosystems). The genotyping cluster was generated with TREECON software developed by University of Konstanz.

Figure 1. Sampling provinces and regions of Phytophthora infestans in China.

Numbers on the map correspond to isolate number finally obtained from different provinces and regions. Three haplotypes, Ia, IIa and IIb

RESULTS

Genotyping analysis (haplotyping and microsatellite analysis)

mDNA haplotype test was a classic genotyping approach of *P. infestans*, which can differentiate four alleles (Ia, Ib, IIa, IIb). Three haplotypes (Ia, IIa, IIb) were found and and all correlated strongly to the region of origin. In Northern China all isolates had the IIa haplotype, in southeast China all isolates had the Ia haplotype, while in southwest China all isolates had the IIb haplotype (Figure 1, Table 1). No Ib strain (US-1 clonal genotype) was found among Chinese isolates.

The eight SSR markers showed a high level of differentiation and finally 43 alleles and 40 genotypes were concluded among Chinese isolates. A genotyping cluster was calculated among Chinese isolates (Figure 2). The occurrence of variable multilocus genotypes was clearly depending on the origin of the isolate and many genotypes are unique to a particular region. The genotyping analysis clearly demonstrates that the current population is not a single clonal lineage. Many genotypes are unique to specific regions. The cluster is divided into three subgroups. The isolates from Northeast, Inner Mongolia Province, Hebei Province and one province Yunnan located in Southwestern China were clustered into Subgroup I, which included the clonal group from the previous study (Guo et al.,

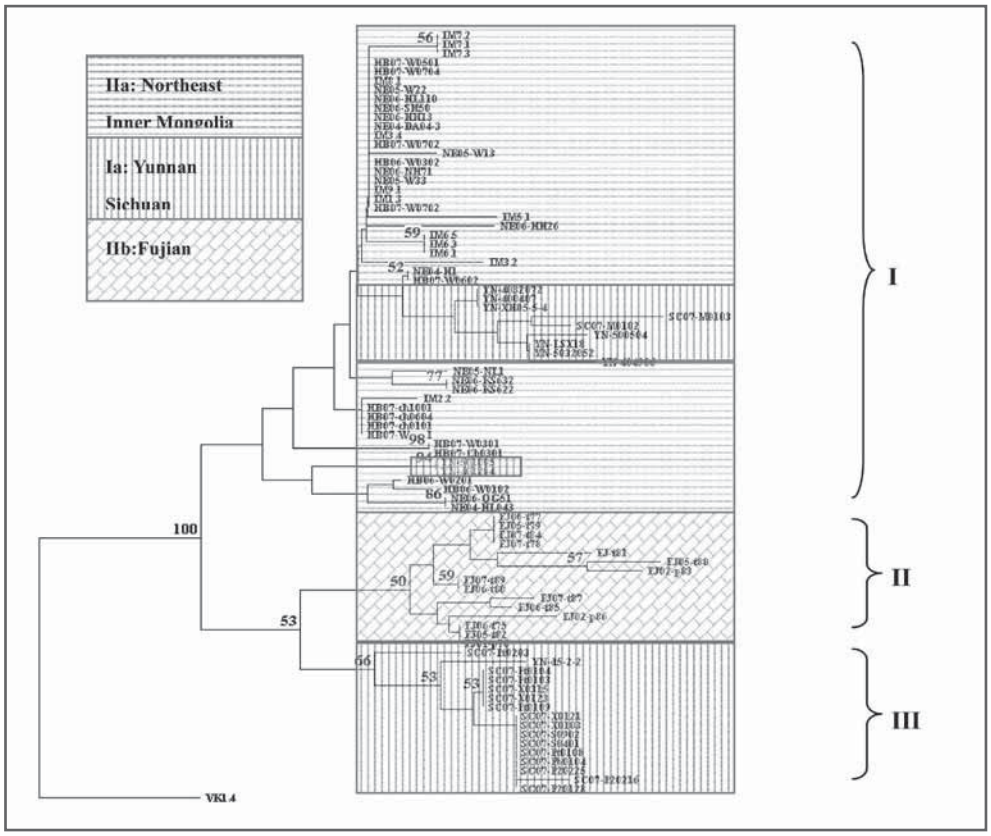

Figure 2. The genotyping clusters by SSR markers.

2009). Three different backgrounds present three haplotypes of isolates. The cluster is divided into three subgroups. Subgroup I contains the isolates from Northeast, Inner Mongolia, Hebei and Yunnan provinces; Subgroup II has the isolates from Fujian province; Subgroup III has the isolates

from Sichuan province. Subgroup II only has the isolates from Fujian province; Subgroup III has the isolates with A2 mating type from Sichuan province and one isolate with A2 mating type from Yunnan Province. Inside one region, genetic structure showed very variable (Figure 2). Isolates from Fujian and Sichuan were clustered to individual subgroups, which showed P.infestans did not crossly migrate between Fujian and Sichuan regions. In Northern regions and Yunnan, it was not surprising that those isolates were together in one subgroup, who were the early potato regions in China and where the potato trade exchange would be more frequent than in other regions.

DISCUSSION

Potato late blight has been through 20 years in China, both mating types were found only since last century, in 1996 (Zhang *et al*., 1996). Few detail reports has been published on whole country-wide *P. infestans* population (Yang, Zhu, and Zhang, 2008), especially modern population structure before this survey. The migration and diversity of *P. infestans* population in China was a mystery, which made very difficult for potato resistance management in China. Since potato agribusiness is stimulated by Chinese government and is developing very fast in recent years, more and more commercial transportation and other potato trade activities are carrying on with national-wide, which could move *P. infestans* around China for instance by infected seed potato's. Meanwhile, potato late blight is not effectively controlled and much more fungicides are used during the potato season in China. The monitoring of *P. infestans* population structure in China is becoming increasingly important to assist forecasting the occurrence of late blight and guide the resistance management in the field.

This survey was performed to unveil the population diversity and forecast the migration of *P. infestans* in China for efficient and long-term potato resistance strategies. Our SSR analysis does not show clear evidence of sexual reproduction. However, more research should be carried out to assess whether the sexual reproduction is a potential threat in China. In the genotyping cluster and haplotype results, Chinese isolates did not belong to the same haplotype or one clonal lineage anymore. In this modern population of *P. infestans*, isolates from Fujian had one unique haplotype, IIb. Ia haplotype was found in Yunnan and Sichuan provinces, but isolates from these neighbor provinces were genetically grouped to individual clonal subgroups, which showed *P.infestans* did not nationally migrate in these provinces. In Northern regions (Northeast, Inner Mongolia and Hebei provinces) and Yunnan, all isolates were together in one subgroup, who were the early potato regions and where the potato trade exchange would be in more frequency than other regions (Yang, Zhu, and Zhang, 2008).

This study gave a full picture of *P. infestans* modern population in China, which will help to understand the population diversity and migration of *P. infestans* in China. For further research on population diversity, we firstly need to set up a storage system for long-term storing the isolates. Many accidents occurred during storing the isolates and in short period all isolates from different years were lost. To learn from those lessons, a long-term storage system with liquid nitrogen would be set up at Institute of Vegetables and Flowers in Beijing. After this general study of *P. infestans* population in China, a deep sampling action to cover all potato regions in China will be successively carried on in coming years. The successive monitoring of population diversity would provide a dynamic trend of *P. infestans* migration in China and help to make the management decision for late blight resistance, and give an advice on policy makers.

The whole sampling work is done with national-wide researchers on potato late blight in China. Based on the current achievements and collaborations, we are going to organize a late blight initiative in China and start up an umbrella project to support of researchers, technology developers

and agricultural knowledge agents to improve short- and long-term resistance management of late blight in China. We started a comprehensive analysis using the most up to date tools and standards but we would like to extend our characterization using more isolates collected from more regions and different years. We also would like to raise awareness on the current *P. infestans* population and the potential risks of latent infection in seed tubers. Therefore we welcome collaboration and seek joined projects to understand the population structure and its application in forecast the occurrence of late blight and guide the resistance management in certain regions similar to what was achieved by Euroblight for Europe.

ACKNOWLEDGEMENTS

The authors want to thank Plant Research International, Wageningen UR (the Netherlands) and Institute of Vegetables and Flowers (China) for financial support, members of Chinese Late Blight Initiates for supplying samples, and the following research assistance: Trudy van der Bosch, Petra van Bekkum and Geert Kessel.

REFERENCES

Chen, Yili, and Dongyu Qu, eds. 2008. Development of potato cropping and processing: Harbin Engineering University Publishing House.

Goodwin, S. B., A. Drenth, and W. E. Fry. 1992. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Curr Genet* 22 (2):107-15.

Griffith, G. W., and D. S. Shaw. 1998. Polymorphisms in *Phytophthora infestans*: four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. *Appl Environ Microbiol* 64 (10):4007-14.

Guo, J., T. van der Lee, D. Y. Qu, Y. Q. Yao, X. F. Gong, D. L. Liang, K. Y. Xie, X. W. Wang, and F. Govers. 2009. *Phytophthora infestans* isolates from Northern China show high virulence diversity but low genotypic diversity. *Plant Biology* 11 (1):57-67.

Qu, Dongyu, Kaiyun Xie, Liping Jin, Wanfu Pang, Chunsong Bian, and Shouguang Duan. 2005. Development of potato industry and food security in China. *Scientia Agricultura Sinica* 38 (2):358-362.

Shattock, R. C., D. S. Shaw, A. M. Fyfe, J. R. Dunn, K. H. Loney, and J. A. Shattock. 1990. Phenotypes of *Phytophthora infestans* collected in England and Wales from 1985 to 1988: mating type, response to metalaxyl and isoenzyme analysis. *Plant Pathology* 39 (2):242-248.

Yang, Zhihui, Jiehua Zhu, and Fengguo Zhang. 2008. Genetic diversity of Chinese isolates of *Phytophthora infestans* revealed by AFLP analysis. *Mycosystema* 27 (3):351-359.

Zhang, Zhiming, Yuqin Li, Shimin Tian, Jiehua Zhu, Jun Wang, and Bofu Song. 1996. The occurrence of potato late blight pathogen (*Phytophthora infestans*) A2 mating type in China. *Journal of Agricultural University of Hebei* 19 (4):62-65.

Zhao, Zhijian, and Zhiming Zhang. 1999. Occurrence of the A2 mating type of *Phytophthora infestans* on potato in Yunnan. *Southwest China Journal of Agricultural Science* 12 (3):1-3.