Highly virulence diversity in a genotypically uniform *Phytophthora infestans* population in Northern China

Jun Guo[1,2,3,*], Theo van der Lee[4], Dongyu Qu[3], Yuqi yao[5], Xiufeng Gong[5], Delin Liang[5], Kaiyun Xie[3], Xiaowu Wang[3], Francine Govers[1,*]

*[1] Laboratory of Phytopathology, Wageningen University, and Graduate School Experimental Plant Sciences, Wageningen, The Netherland; [2] College of Plant Protection, Northwest A&F University, Yangling Shaanxi 712100, China; [3] Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China; [4] Plant Research International, Wageningen University and Research Center, Wageningen, The Netherlands,[5] Inner Mongolia Zhengfeng Seed Potato Company LTD, Huhhot 010031, China. *E-mail: guojunwgq@nwsuaf.edu.cn; francine.govers@wur.nl.*

Abstract

Phenotypic and genotypic characteristics of 48 *Phytophthora infestans* isolates, collected in five provinces in Northern China in the period 1997 to 2003, were determined and compared with reference isolates from Southern China and other part of the world. Characterization included mating type, virulence, mitochondrial DNA (mtDNA) haplotype and DNA fingerprinting patterns based on simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP). All isolates from Northern China had the A1 mating type, mtDNA haplotype IIa and an identical SSR genotype (designated as SG-01-01) that differed from the SSR genotypes found in all the reference isolates. In contrast, the virulence spectra were highly variable and virulence to all resistance genes represented in the standard differential set (R1 to R11) was found. Also AFLP analysis revealed some diversity; eight different AFLP genotypes were found that could be grouped in two major clusters. This study shows that the *P. infestans* population in Northern China seems to represent one clonal lineage that is different from the 'old' US-1 lineage that dominated the worldwide *P. infestans* population prior to 1980. The occurrence of many different races within this uniform population is discussed in the framework of recently gained insights in the molecular determinants of avirulence in potato-*P. infestans* 'gene-for-gene' interactions.

Results and Analysis

- The 48 isolates from Northern China all had the A1 mating type; no A2 mating type and self-fertile isolates were found.
- The virulence spectrum in the isolates was highly variable. Of the 43 isolates that were tested six could infect all eleven differentials and one could infect only one of the eleven. A total of 21 different races was distinguished, of which race 3.4.7.10 (18.6%), race 1.2.3.4.5.6.7.8.9.10.11 (14.0%) and race 3.4.6.7.10 (11.6%) were the most frequent. In Inner Mongolia, eighteen of the 21 races were found.
- Genotyping of the mtDNA revealed that all isolates from Northern China have the IIa haplotype (Figure 1). The other three haplotypes were not detected. From the reference isolates only the Ecuadorian isolate EC3417 had the IIa haplotype. The others are either IIb (YN-B13), Ib (VK1.4 and IPO-0) or Ia (the other eight).

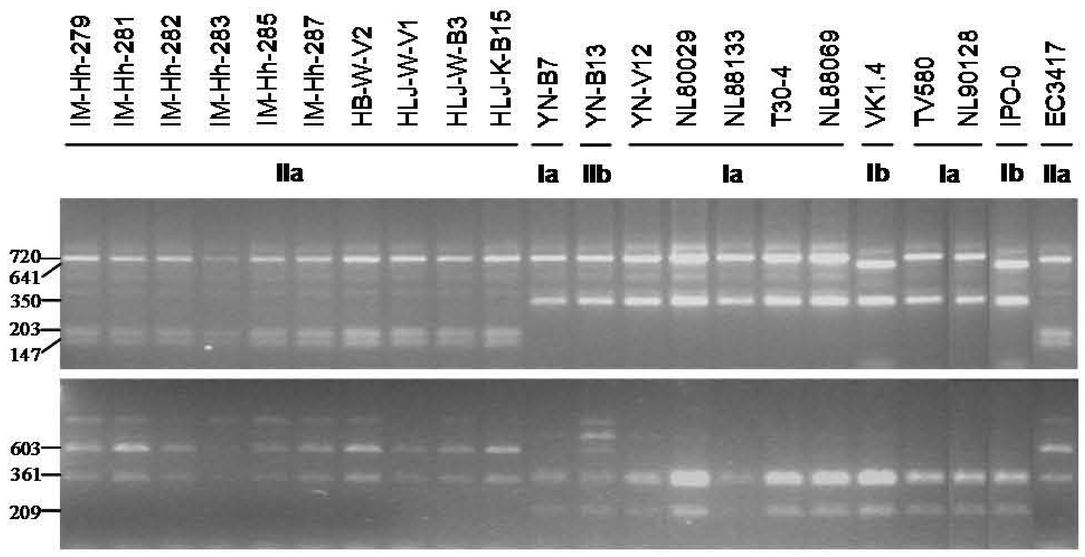

Figure 1. The mtDNA haplotype of ten *P. infestans* isolates from Northern China and twelve *P. infestans* reference isolates. The mtDNA was amplified with P2 primers or P4 primers and digested with *Msp*I and *Eco*RI, respectively. The sizes of the restriction fragments (in bp) are indicated on the left.

- All isolates from Northern China had the same SSR genotype SG-01-01 which was not found for any of the reference isolates (Figure 2). Of the reference isolates the three from Yunnan Province shared a unique SSR genotype, SG-02-01. The remaining nine reference isolates each had their own unique SSR genotype. Among the reference isolates there is no obvious association of the SSR genotypes with mating type, mtDNA haplotype or geographic origin of the isolates.

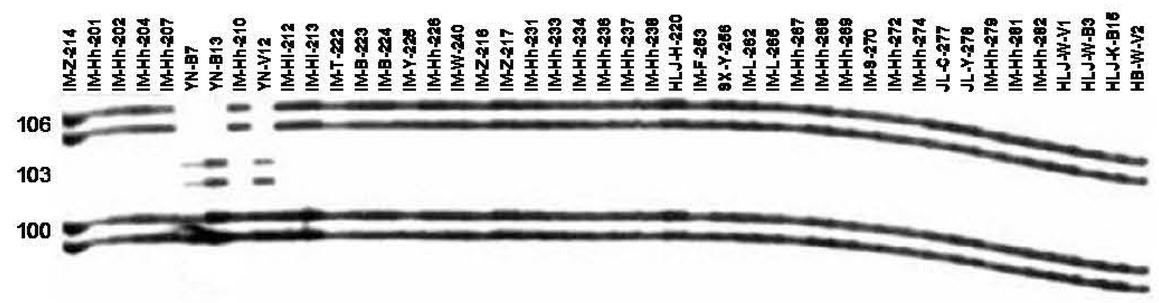

Figure 2. SSR genotyping of *P. infestans* isolates from Northern China and three reference isolates from Yunnan Province (with prefix YN) with primer pair PiGCA-0012. The numbers on the left indicate the three alleles that can be distinguished with PiGCA-0012.

- AFLP analysis revealed some diversity, eight different AFLP genotypes were found that could be grouped in two major clusters, I and II (Figure 3). Clustering of the AFLP genotypes could not be associated with the collection year or the geographic origin of the isolates.

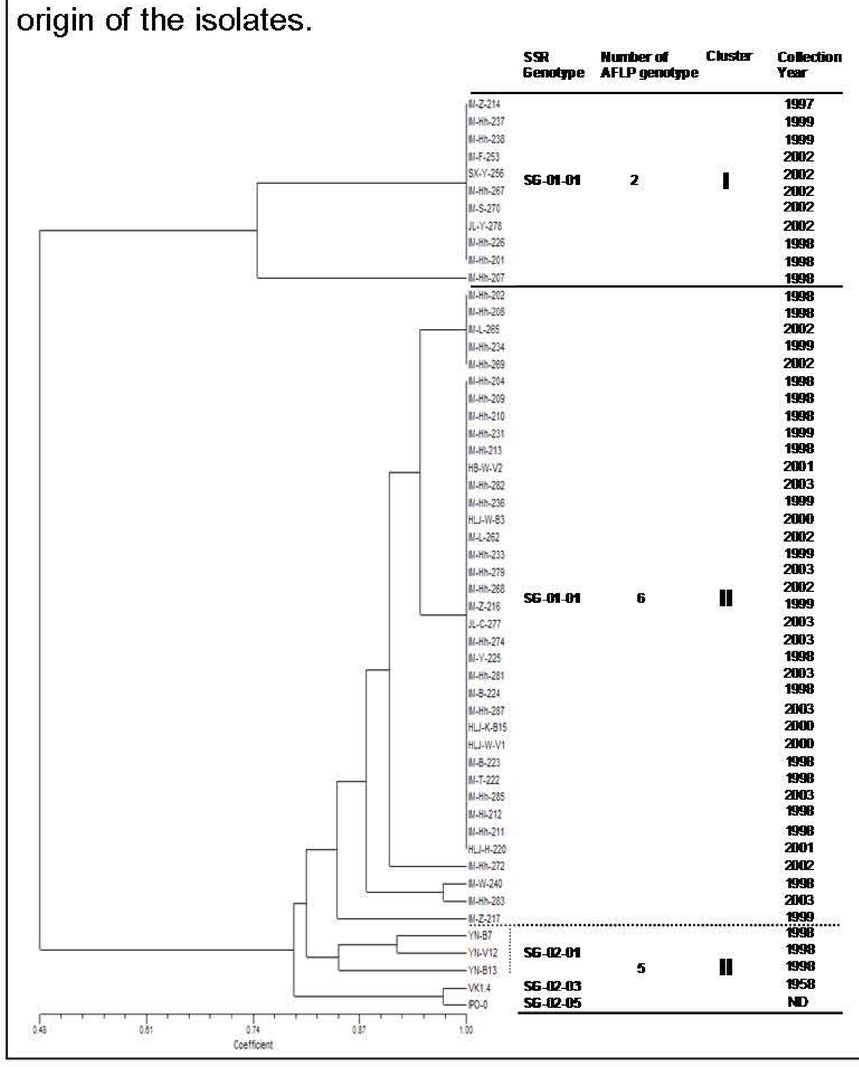

Figure 3. Cluster analysis of 48 Chinese *P. infestans* isolates that have the same SSR genotype SG-01-01 and five reference isolates. The tree was inferred by bootstrap analysis of 35 AFLP markers. ND: no data.

Introduction

- Potato is an important crop in Northern China.
- Potato late blight become more serious in these regions in recent years.
- Hardly any molecular genotyping of Chinese *P. infestans* isolates has been reported in an international scientific journal.
- The main aim is to perform a more comprehensive survey of the *P. infestans* population in Northern China, in particular in Inner Mongolia, and to compare that population with reference isolates including a few isolates from Southern China.

Materials and Methods

- 48 *P. infestans* isolates from Northern China, 3 (YN-B7, YN-B13, YN-V12) from Yunnan and 9 other reference isolates (NL80029, NL88133, T30-4, NL88069, Vk1.4, TV580, NL90128, IPO-0, EC3417).
- Phenotypic characterization : Mating type determination, Virulence testing.
- Genotypic characterization: SSR (Simple Sequence Repeat) analysis, AFLP analysis (primer combinations P20H25 and P22H23) and Mitochondrial DNA haplotyping.