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Characterization of *Phytophthora infestans* population in Chile

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SUMMARY
Today, Late blight is the most important disease in potato in Chile, being an epiphythotic disease since 2005, showing high incidence and severity, causing more than 50% of yield reduction, depending of the season weather conditions. Studies performed since 2003 to 2011 on *P. infestans* population in southern Chile revealed genetic changes, although still it is an A1 mating type. But until the season 2004-05 the population was susceptible to metalaxil (<3 ppm EC50), today, the population is highly resistant to metalaxil (> 100ppm EC50), shows pathotypes highly complex and has different SSR patterns than the previous population. Moreover, the mitochondrial DNA analyses showed different haplotypes, while the first collection was Ib, the current is Ia.

KEYWORDS
Late blight, *Phytophthora infestans*, characterization of pathogen population

INTRODUCTION

Phytophthora infestans (Mont.) De Bary, the causal agent of late blight, like the potato, its host, has been able to adapt to different climates and latitudes (Garlick *et al.*, 2002). New biotypes have arisen in the last decades making the control more difficult (Fry and Goodwin, 1997).

P. infestans is heterotalic, with two mating groups A1 and A2. The group A1 was predominant worldwide. The group A2 was reported only in Mexico until late 80's. At the end of the 20th century *P. infestans* migrated from Mexico, thus increasing the genetic diversity of populations of this pathogen in most of the continents. The first new genotypes were detected in Europe, then in South America, Africa and Asia and finally USA and Canada. This group has becoming predominant and more aggressive (Stevenson *et al.*, 2001). In South America the group A2 has being described in Argentina, Bolivia, Brazil, Ecuador, and Uruguay (Adler *et al.*, 2002; Crissman and Lizárraga, 1999).

The first reports of disease caused by *P. infestans* in Chile are from the 50's and it is thought that it came from Argentina (Anónimo, 1951). This had a great impact on the potato crop where most varieties being cultivated in that period almost disappeared, what actually happened to the red

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Corahila potato variety. Since then few studies have being done to characterize the late blight population. All the isolates described in a study done by INIA and NDSU in the northern Chile were found to be the group A1/US1 of several pathotypes of low aggressiveness. The isolates were highly resistant to Mefenoxam (> 300 ppm), because of the continuous use of Ridomil (Secor, 2003). Previously, Fernandez (1979) studied *P. infestans* virulence in southern Chile populations, describing complex pathotypes able to infect even five plant differentials.

Today, Late blight is the most important disease in potato in Chile, being an epiphythotic disease, showing high incidence and severity, causing more than 50% of yield reduction, depending of the season weather conditions. Since 2003, The Agricultural Research Institute of Chile (INIA), associated with public and private institutions, are performing studies to Implement an Integrated pest management for late blight based on a forecasting system (Acuña, *et al*., 2009). One of the main objective of this project is to characterize and monitor the *Phytophthora infestans* population associated to the potato crop in southern Chile

MATERIALS AND METHODS

P. infestans monitoring has been performed through the seasons 2003-04 until 2010-11 in the potato production area of southern Chile determining mating types, avirulence genes, metalaxil resistance, DNA polymorphism (SSR) and mitochondrial haplotypes.

Collection of 250 *P. infestans* isolates were performed during years 2003 to 2005 and 259 during 2006 to 2011, from lesions on potato plants and tubers, at southern Chile from Araucania region (parallel 39° S) and Chiloe island (parallel 43°S).

A piece of leaflet or tuber with symptoms was put under a potato slices of Yagana cultivar. Then, the samples were incubated at 18°C for 7 days. After that, four pieces of infected tissue were transferred to a Petri plate containing rye B agar, amended with antibiotics (Ampicillin) (Forbes, 1997). The isolates were incubated during 4 to 7 days at 18°C in darkness. *P. infestans* isolates were transferred to Rye B media and maintained at 18°C in darkness.

Because A2 mating type has not been described in Chile, the isolates were evaluated for mating type at the Agricultural National Service (SAG) in Santiago, Chile. The test was conducted by placing an agar plug containing mycelium on the edge of two rye B agar plate pairing with a similar size agar plug of known A1 and A2, one for each plate. After 15 days at 15°C, the plates were examined for oospore production (Tooley *et al*., 1989, Miller *et al*., 1998 and Dorrance *et al*., 1999).

In vitro metalaxyl sensitivity was assessed by comparing *P. infestans* radial growth on Rye B media amended with 5 different concentrations of metalaxil to a growth on metalaxil free control (Deahl, 1993). The test for each isolate and metalaxil concentration (0, 0.1, 1.0, 10, 100 ug/ml) was performed with 5 mm plug of a 10 days old growing colony placed in the center of a 9 cm Petri dish with the corresponding amended media. After 10 days of incubation at 18°C on darkness, two perpendicular measurements were taken for each plate. The percentage of relative growth on amended media versus control was scored and the EC50 was calculated as described by Miller *et al*. (1998).

The virulence assay was carried out by inoculation of detached leaflets of a differential set of plants with the 11 known major R genes for resistance. Craigs Royal cultivar was used for Race R0. Differentials were obtained from NDSU, originally from the Scottish Crop Research Institute, Scotland. Race determination was based on compatible host-pathogen reaction seven days after inoculation with a 2 x104zoospores/ml (Miller *et al*., 1998). Lesions were read on a scale 0=no symptoms, 1= hypersensitive reaction, 2= necrosis no sporulating, 3= necrosis and sporulating lesion.

A DNA polymorphism among isolates was established using the SSR Pi02, Pi04, Pi16, Pi26, Pi33, Pi56, Pi66, and Pi70. Primer sequences and PCR protocol for SSR were facilitated for Dr. David Cooke from the SCRI. Amplified PCR products were separated in standard DNA sequencing PAGE and silver stained method was used to visualize the DNA fragments.

RESULTS AND DISCUSSION

Results send by the SAG laboratory described all the isolates as A1 mating type for all the evaluated crop seasons.

However, the *P. infestans* population until the season 2004-05 was susceptible to metalaxil with an EC50 less than 3ppm, highly complex pathotypes, but mainly with 2, 4 and 5 avirulence genes. Moreover, the most frequent genes were 10 and 11, present in 93% of the population (Acuña *et al.*, 2007). However, the 2006 to 2011 collection was metalaxil resistance with an EC50 over than 100 ppm and a relative growth on Rye B media amended with 10 ppm of metalaxil over a 60% (Figure 1). It shows complex pathotypes with mainly 7, 8 and 9 avirulence genes and with high frequency of R1, R3, R5, R7, R8, R10 and R11 (Figure 2 and Table 1).

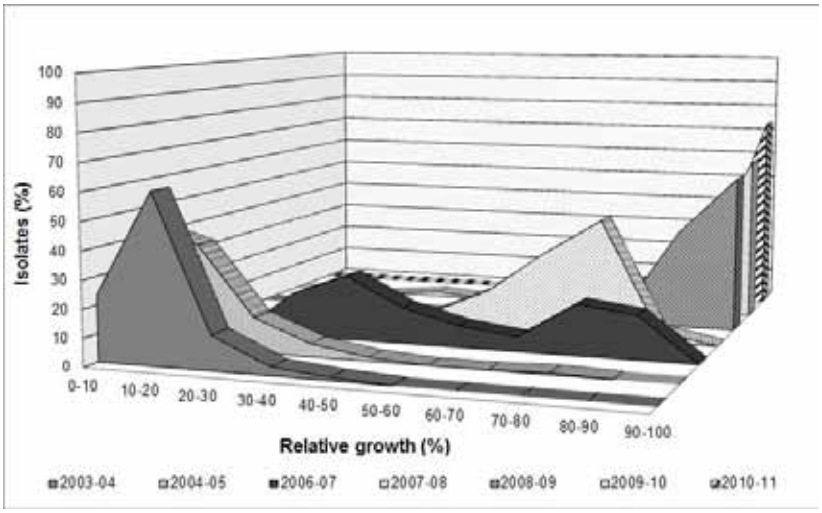

Figure 1. Relative growth of P.infestans isolates collected since potato growing season 2003 to 2011, cultured on Rye B media compared to isolates grown on Rye B media amended with 10 ppm of metalaxil.

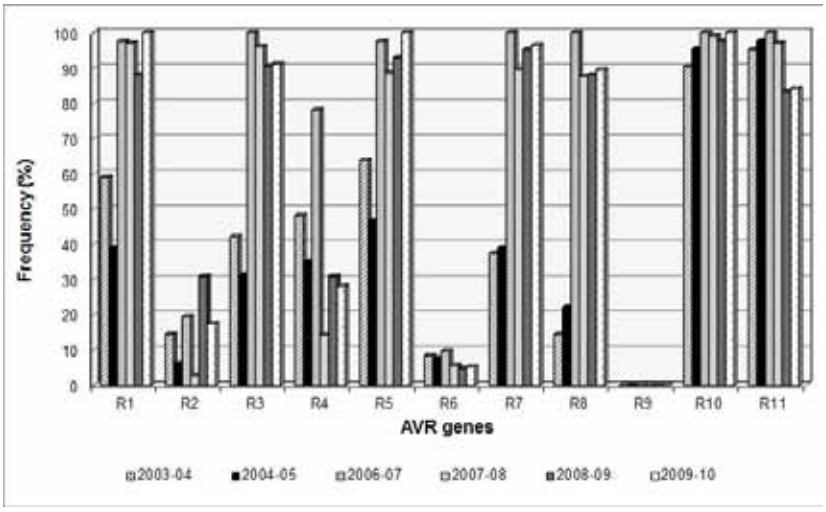

Figure 2. Avr gene frequency in the P. infestans population since 2003 to 2011 from the southern Chile.

Table 1. Pathotype complexity of P. infestans population for the season 2003 to 2011 at southern Chile.

AVR genes per isolates	Isolates per season (%)					
	2003-04	2004-05	2006-07	2007-08	2008-09	2009-10
1	3,6	0,0	0,0	0,0	0,0	0,0
2	14,5	22,9	0,0	0,0	2,4	0,0
3	8,4	13,0	0,0	3,8	2,4	0,0
4	16,9	20,6	0,0	1,9	2,4	0,0
5	25,3	16,0	0,0	2,9	2,4	1,8
6	13,3	18,3	2,4	13,3	9,5	14,0
7	4,8	6,1	22,0	62,9	42,9	52,6
8	10,8	1,5	51,2	13,3	31,0	29,8
9	2,4	1,5	19,5	1,0	7,1	1,8
10	0,0	0,0	4,9	1,0	0,0	0,0
11	0,0	0,0	0,0	0,0	0,0	0,0

Additionally, this last population shows different SSR pattern than the previous population. According SSR pattern, 5 genotypes were distinguished during the collecting seasons 2003 to 2005, but one of them showed 70% of the frequency (Acuña *et al*., 2010). Moreover, at the collection 2006 to 2011, two new genotypes were present, one of them with more than 90% of the frequency (Figure 3). This last genotype was described for the first time during the season 2005/06, where only one isolated was found. Today it is the more predominant. Among the genotypes most polymorphisms were detected at Pi02 and Pi16 loci.

Additionally, the mitochondrial DNA analysis revealed a new haplotypes since 2006, then the previous population was Ib and the new one is Ia (Figure 4) (Acuña *et al*., 2011).

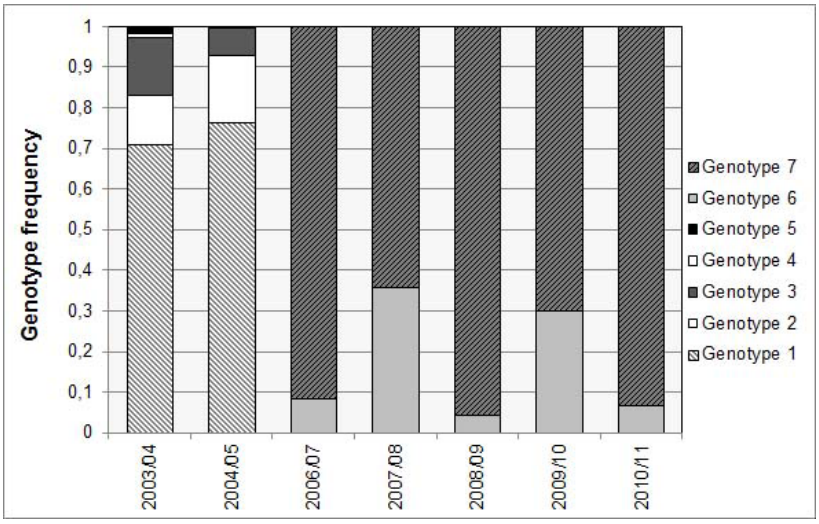

Figure 3. Genotype frequency of P. infestans population in southern Chile using SSR analysis.

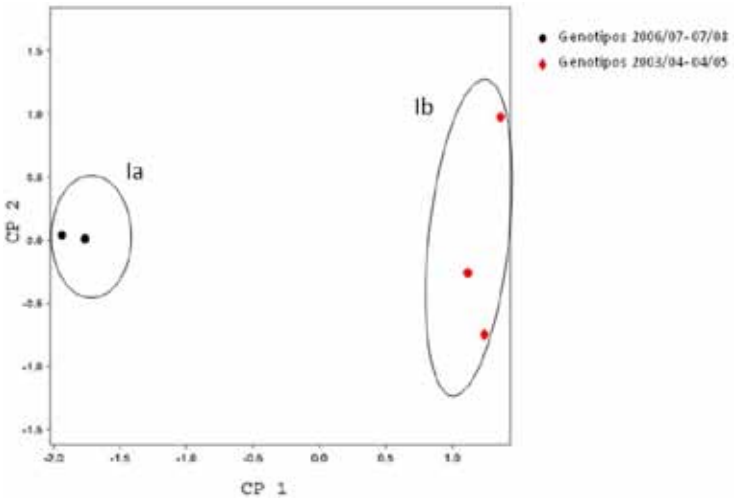

Figure 4. Mitochondrial DNA analysis for P. infestans isolates from the season 2003 to 2008 at southern Chile.

CONCLUSIONS

The epidemiology of late blight on potato in Chile has changed since 2005, being an epiphythotic disease when the weather conditions are favorable for the disease. The main possible reason is that the *P. infestans* population is showing genetic changes, although still it is an A1 mating type. But until the season 2004-05 the population was susceptible to metalaxil (<3 ppm EC50), today, the population is highly resistant to metalaxil (> 100ppm EC50), shows pathotypes highly complex and has different SSR patterns than the previous population. Moreover, the mitochondrial DNA analyses showed different haplotypes, while the first collection (2003-05) was Ib, the current is Ia (2006-08)

These results showed a change in population characteristic, due to, probably, an introduction of a new population or a selective pressure, because of the management or weather conditions, on a previous population in low frequency, associated to cultivated or wild *Solanum*s. Future steps in this research will include *P. infestans* isolates associated to other *Solanum* species in Chile, both cultivated and wild, and considering other worldwide databases information.

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