

Genotypic variation of *Phytophthora infestans* populations in Argentina

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KEYWORDS

Potato late blight, Argentina, population genetics, simple sequence repeats, mating type, mitochondrial DNA haplotype, genotyping

INTRODUCTION

Potato is an important staple crop in Argentina and their production fluctuates over the years, reaching in 2010 almost 2 million tonnes (FAOSTAT, 2012), for domestic market. Spunta is the most important potato variety with more than 70% of the national production for fresh market (Rodríguez Quijano, P., 1989; private and official estimates not published, 2011). Based on production data, recent statistics positioned Argentina in 2010 in the 30th place in the world and fourth in Latin America (FAOSTAT, 2012). Potato is grown almost continuously throughout the year, alternating between different production areas. 80% of production is concentrated in the Provinces of Buenos Aires, Córdoba and Santa Fe, the remainder being distributed between the Provinces of Mendoza and Tucumán and the rest of the country. The highest yield in the country was observed in the Southeast region of the Province of Buenos Aires (SEBAP).

Potato late blight caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is the greatest biotic limitation of production worldwide and is a major threat to food security and poverty reduction. It is responsible for global losses of over 5 billion US\$ (Anderson *et al.*, 2004; Pennisi, E., 2010).

The development of the disease is highly dependent on weather conditions (Agris, G. N., 2005). In SEBAP, where is concentrated the highest potato production, the weather conditions are conducive to the disease development, reporting losses during the period 1986-2005, without chemical control, over 40% for commercial tubers and over 35% in total yield (Mantecón, J. 2009).

In order to advance in the knowledge of the pathogen populations present in Argentina, we characterized by different phenotypic and genotypic markers, 87 isolates of *P. infestans* collected during 1992-1995, 1997-99 (Distel and Huarte, 2000) and 2009-10 and from different potato growing areas of the country.

MATERIALS AND METHODS

87 isolates of *P. infestans* were collected from the main potato areas of Argentina: SEBAP, Tafí del Valle (Tucumán) and Córdoba Province. The collection of isolates was done during three different periods: 1992-95 (45 isolates), 1997-99 (24 isolates) and 2009-10 (18 isolates).

P. infestans cultures obtained by isolation from late-blight lesions (from potato leaves, tubers and stems) were done on potato tuber slices and then transferred onto Rye A medium and Pea Broth medium (Perez and Forbes, 2008).

The isolates were examined for mating type, mitochondrial DNA haplotypes, specific virulence using standard techniques (Cooke *et al.*, 2003).

For genotyping of *P. infestans* isolates, DNA was extracted using a commercial kit and the multiplex amplification was performed as Lees *et al.* (2006) suggest with 12 microsatellites markers: SSR3, SSR11, SSR4, SSR6, Pi63, SSR2, PiG11, Pi70, Pi4B, SSR8, D13, Pi04. The isolates of 2009-10 and a subgroup of 1997-99 were genotyped. Peak size and quantification data generated using GeneMapper 2.7 (Applied Biosystems).

RESULTS AND DISCUSSION

Phytophthora infestans is a heterothallic organism that has two mating types A1 and A2. In relation to the population of *P. infestans* in Argentina, their structure varied significantly during these periods. For the isolates collected during 1992-95. Both mating types were detected with an average of 25% for A1 and 75% for A2 mating types (Distel and Huarte, 2000). For the following period the mixed population of A1 - A2 was displaced rapidly towards A2 mating type. This trend was apparently conserved during 2000 and 2003 (Andreu *et al.*, 2005). Isolates of *P. infestans* from Uruguay collected between 1998 and 1999 showed similar profile to that found in Argentinian *P. infestans* populations (Deahl *et al.*, 2003). On the other hand, newer isolates collected between 2009-10 potato crop season showed a new variation of the population of *P. infestans* towards predominant A1 mating type and with Ia Mitochondrial DNA haplotype. This trend is observed in preliminary studies conducted with *P. infestans* isolates from 2010-11 season.

On the other hand, diversity for virulence and complexity of races increased greatly during the mentioned periods (Table 1). Isolates collected throughout 1992-95 showed only 4 virulence factors (1, 4, 10, 11), increasing the number of factors to 6 during 1997-99 (1, 3, 4, 8, 10, 11). For the last period (2009-10), all virulence factors were present in at least one of the isolates tested (Fig. 1). Virulence factors 2, 5, 7 and 9 were only found in newer isolates. In addition, physiological races of *P. infestans* had increased the number of virulence factors in time. The distribution of races in the different potato growing areas revealed that Tafí del Valle (Tucumán Province) isolates had the highest number of virulence factors (10 virulence factors) and old isolates from Balcarce (SEBAP) had the lowest complexity (2 virulence factors).

Genotyping was performed in isolates collected during 2009-10 based on microsatellites to study genetic variation in pathogen populations and to identify the impacts of migration on the evolution of new strains. For the characterization some isolates collected during 1997-99 were included. Oldest isolates (1997-1999) showed a divergent profile from the new population, as well as on the type of mtDNA haplotype. Recent isolates showed Ia haplotype and the oldest ones showed IIa and Ia haplotypes.

Although more recent isolates have shown high homogeneity for most of SSR markers, the PiG11 marker allowed to clearly distinguished those isolates from the area of Tafí del Valle (Tucumán Province) from those collected in Córdoba and SEBAP in the same period.

Currently, the genotyping of new *P. infestans* isolates that were collected during the 2010-11 and 2011-12 season in the SEBAP is under analysis, in order to follow the dynamics of the *P. infestans* populations in details. All phenotypic and genotypic characterization tools are implemented to confirm the trend shown with the isolates collected in the last period, 2009-10. The information of changes occurred with *P. infestans* populations in Argentina will render a valuable tool to develop durable resistance to potato Late Blight.

CONCLUSIONS

The structure of *P. infestans* populations in Argentina have undergone significant changes over the last 20 years, showing variations in the predominant mating type populations, increasing the degree of genetic variability, the diversity of races and number of physiological factors virulence of the pathogen. The results of mitochondrial haplotype analysis indicated that oldest population of *P. infestans* was characterized by a combination of Ia and IIa haplotypes, and turned predominantly to Ia haplotype in the newer population. On the other hand, the mating type of the populations has also shown high dynamic performance, detecting both mating types in the period 1992-95, that was shifted to A2 during 1997-99. This trend appears to have remained during the early years of 2000. Recent isolates showed the predominance of type A1.

Genotyping of recently isolates, which included some isolates of the period 1997-99 as reference of this population, showed divergent profiles in both populations. The screening of isolates collected during 2010-11 and 2011-12 seasons is still to be finished, but preliminary results support that trend. Characterization of a much greater number of isolates will help to confirm the trend shown in the population in 2009-10 and also provide an updated picture of the structure of *P. infestans* population in Argentina.

REFERENCES

- Agrios, G. N. 2005. Plant Pathology (5th edition). Elsevier-Academic Press. San Diego, CA. 922p.
- Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R., and Daszak, P. 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends Ecol Evol.* 19:535-544.
- Andreu, A. B., Caldiz, D. and Forbes, G. A. 2005. Phenotypic Expression of Resistance to *Phytophthora infestans* in Processing Potatoes in Argentina. *Am. J. Pot. Res.* 87:177-187.
- Cooke, D. E. L., Ypung, V., Birch, P. R. J., Toth, R., Gourlay, F., Day, J. P., Carnegie, S. and Duncan, J. M. 2003. Phenotypic and genotypic diversity of *Phytophthora infestans* population in Scotland (1995-97). *Plant Pathology* 52, 181-92.
- Deahl, K.L., Pagani M.C., Vilaro, F.L., Perez, F.M., Moravec, B. and Cooke, L.R. 2003. Characteristics of *Phytophthora infestans* isolates from Uruguay. *European Journal of Plant Pathology* 109: 277-281.
- FAOSTAT, <http://faostat.fao.org/>
- Huarte, M. A. and Distel S. 2000. Comparación en el tiempo de poblaciones de *Phytophthora infestans* en Argentina. *Memorias del XIX Congreso de la Asociación Latinoamericana de la Papa, Cuba, SV-9, Page 14.*
- Lees, A. K., Wattier R., Shaw, D. S., Sullivan, L., Williams, N. A., Cooke, D. E. L. 2006. Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. *Plant Pathology*, Volume 55, Issue 3, pages 311-319.
- Mantecón, J. D. 2009. Importance of potato late blight in Argentina, and the effect of fungicide treatments on yield increments over twenty years. *Ciencia e investigación agraria* 36(1): 115-122.
- Pennisi, E. 2010. Food Security: Armed and Dangerous. *Science* 327: 804-805.
- Pérez, W. and Forbes, G. 2008. Manual técnico: El tizón tardío de la patata. Centro internacional de la patata (CIP).
- Rodriguez Quijano, P. 1989. La selección de tecnología por los agricultores. El caso de las variedades de patata en el Sudeste de la Provincia de Buenos Aires. Tesis M. Sc. Escuela para Graduados Facultad de Agronomía UBA.

Table 1. Diversity of virulence factors present in *P. infestans* populations based on isolates collected during 1992-95, 1995-97 and 2009-10.

Virulence factors	<i>P. infestans</i> isolates *					
	1992-95	%	1997-99	%	2009-10	%
Present	1	100,00%	1, 10, 11	100,00%	1, 3, 4, 11	88,89%
	10	62,20%	3	93,80%	10	66,67%
	11	55,60%	8 y 4	81,30%	2	55,56%
	4	51,10%			5	44,44%
					6, 7, 8	22,22%
				9	11,11%	
Absent	5	100,00%	6	100,00%		
	6	100,00%	9	100,00%	-	
	8	100,00%				
	9	100,00%				

* (years of collection- % of individual virulence factor)

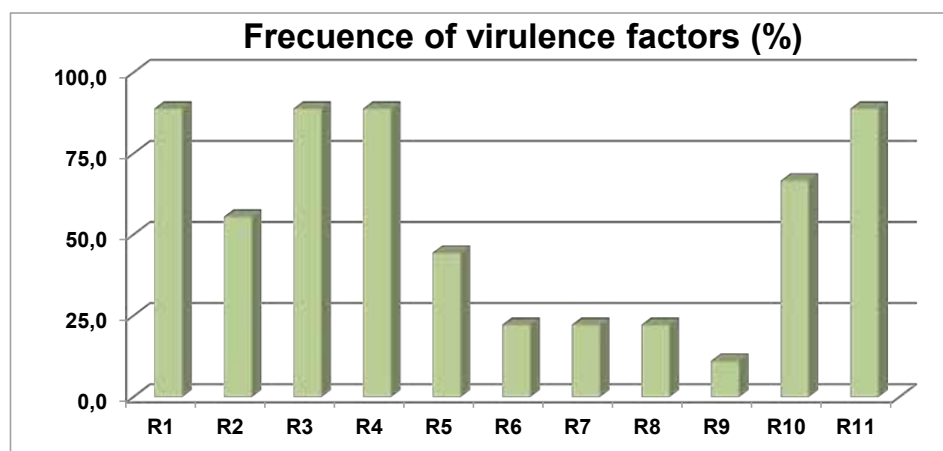


Figure 1. Average gene frequencies in *P. infestans* populations from 2009-10 season.