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New Genomic approaches to study Phytophthora populations

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The center of origin of potato and *P. infestans* is Latin America, where the pathogen co-evolved with a large diversity of Solanaceous species. The first historical tragedy associated with *P. infestans*, the Irish potato famine, occurred 170 years ago. Even today, problems associated with *P. infestans* remain the main threat that affects potato and tomato crops around the world. In the decade of the 90's, late blight returns to be a global problem, causing epidemics in the potato production systems, due to migration and changes in the characteristics of the populations of the pathogen, which presented greater aggressiveness and resistance to fungicides causing loss of strength in commercial varieties. In Europe during severe blight years, up to 25 sprays were used per season in some countries (Hansen et al., 2009), and fungicide insensitivity was evolving in parts of Europe (Nielsen, 2014). Haverkort et al. (2009) estimated globally annual costs of € 5.2 billon including the costs of control measures. Late blight is considered a re-emerging disease encouraged by the increasing globalization of trade and climatic change. The disease has reached epidemic proportions in North and South America, and Europe due to the development of resistance to the fungicide metalaxyl in populations of the pathogen and the widespread occurrence of new more aggressive genotypes that are difficult to control (Kadish et al., 1990; Fry, 2015).

Nowadays, newly available capacity enables much greater precision in late blight management.

Potato is an important staple crop in Argentina. The most important losses of the crop are due to late blight control measures and losses of yield and quality associated with it. The South East of the Buenos Aires Province (SEBA), where the highest yields are obtained, the agro-ecological conditions are very conducive to late blight development. Currently, it is primarily controlled through frequent fungicide applications. Spraying programs are based on more or less fixed intervals, starting as early as 30 days after planting. Spraying frequencies may range from 7 - 10 days, depending on possible cultivar resistance, weather conditions, growth stage and active ingredient (Mantecón, 1998, 2000). As in many other countries, food companies and consumers are looking for more sustainable management and production technologies.

At National Agricultural Technology Institute (INTA), our research is focused on studies of the pathogen, its epidemiology and ecology, genetic improvement of the host and integrated pest

management. At the Mycology and Bacteriology Lab of Balcarce Research Station we developed, assessed and analyzed the impact of PhytoAlert, a decision support system (DSS) to control LB regarding disease control effectiveness, production costs and environmental impact during four consecutive growing seasons in SEBA.

PhytoAlert DSS was used to predict the critical moments for the development of late Blight since 2010 and the implementation of a preventive control strategy based on prevailing weather conditions (measured and forecasted), host resistance, and the degradation of fungicide.

PhytoAlert DSS improved the control of Late Blight by reducing fungicide use up to 50% and economic losses up to 47% and achieving a lower environmental impact (up to 48%) compared to a Calendar-based control system applied in the area (Lucca & Rodriguez, 2015).

We were also interested in epidemiological studies of *P. infestans* populations in Argentina and the region.

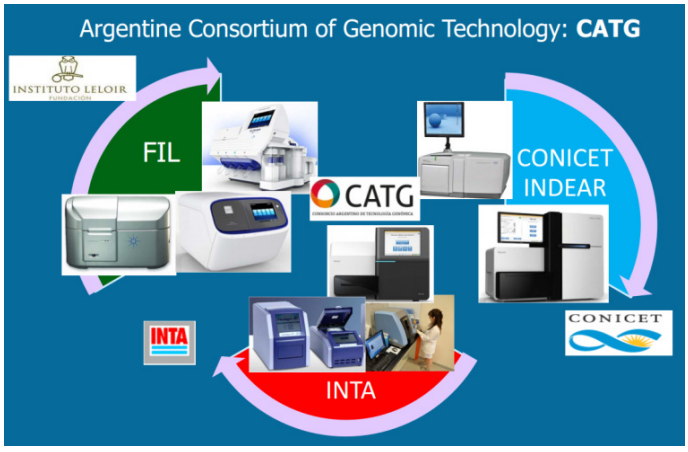
Genotypic diversity in *P. infestans* was historically assayed with several genotyping tools, from allozymes (Tooley & Fry, 1983), restriction fragment length polymorphisms (RFLP) (Goodwin et al., 1992), mitochondrial haplotypes (Danies et al., 2014, Carter et al., 1990), and microsatellites or simple sequence repeats (SSRs) (Li et al., 2013).

Recently GBS (genotyping by sequencing) (Elshire et al., 2011; Hansen et al., 2016) or gene sequencing (Dong et al., 2014, Goss et al., 2014) approaches have been used.

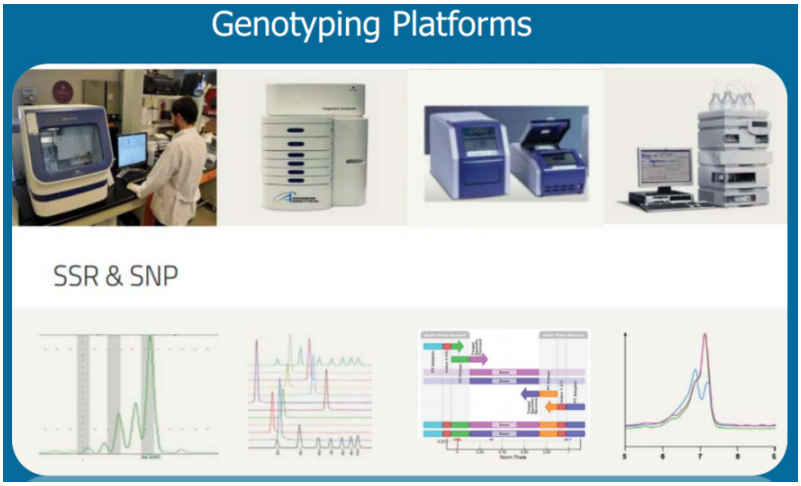
The development of Next-Generation Sequencing technologies (NGS) at reduced cost, allowed the use of these high throughput tools to answer important biological questions. Technological advances allow the generation and/or interrogation of massive amounts of genotype data and also to sequence genome of entire collections of microorganism.

In this sense, the Mycology and Bacteriology Lab (EEA INTA Balcarce) is working collaboratively with the Genomic Unit of Biotechnology Institute of INTA to carry out SSR genotyping and new available genomic strategies that can be applied to the study Phytophthora populations.

Genomic Unit (UGB.nCATG) of INTA is node of Argentine Consortium of Genomic Technology (CATG), a core laboratory oriented to the analysis of molecular markers and DNA sequencing that responds to internal and external demands of the public and private sectors, including the agricultural and livestock sectors, forestry, health, energy, environment, fishery, forensic, anthropological and judicial, in the areas of diagnosis, molecular epidemiology, prospection / discovery, breeding, regulation and control.



Installed capacities at INTA and accumulated experiences allow us to develop a wide spectrum of applications in the area of genomics using new technologies. UGB has specialized in genotyping techniques mainly targeting the breeding and molecular epidemiology sectors, focusing on target gene sequencing, complete sequencing of microbial, mitochondrial and plastid genomes, transcript sequencing and genotyping by sequencing.
The laboratory has ISO 17025 accreditation since 2012, being the only one in the country that has adopted quality standards for services in the area of genomics.



Training and outreach activities between partner countries, in particular for Late blight studies, with Tizón Latino Network are carrying out to share expertise and technical capacity in specific areas. Trainees carry out activities as learning about technologies, assay design, library construction and results analysis. Researchers specializing in the topics to be addressed advise them in the different stages of assay.
The Institute of Biotechnology also has a Bioinformatics Unit that provides the necessary infrastructure for data storage and for population dynamic studies.
The Bioinformatics Unit and its associated nodes are not only generating but also have experience in the processing and administration of previous data. The Bioinformatic Unit offers courses and internships and develops pipelines and free access bioinformatics tools for the analysis of NGS data. The close interaction between both Units allows improvements in protocols and adjustment of data analysis to obtain high quality results.

Our first epidemiological study of *P. infestans* in Argentina was carried out in SEBA by sampling, extraction of ADNg in FTA cards and genotyping of populations with an internationally agreed panel of 12 microsatellite markers (Li et al., 2013). Genetic studies showed that all isolates collected since 2007 to present belong to genotype 2_A1, although allelic variants were observed in the material evaluated.

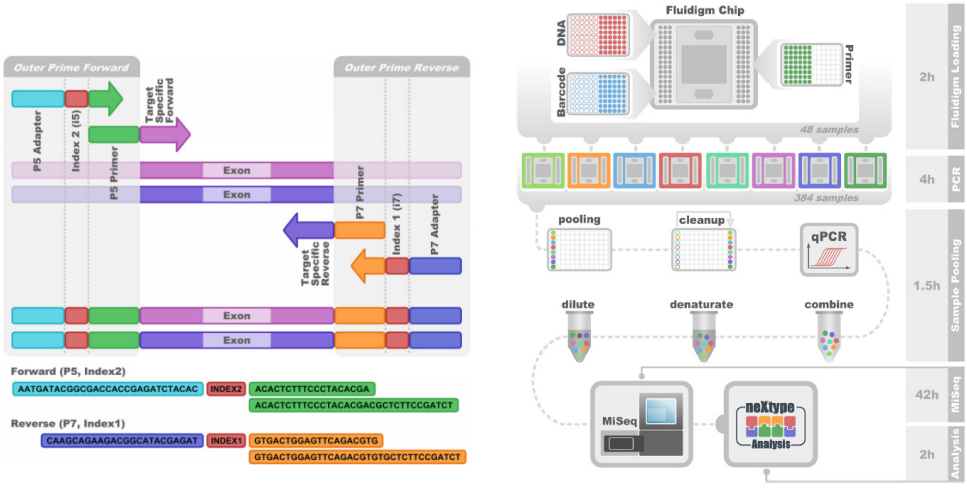
A Latin American late blight network called Red Tizón Latino was launched in Bogotá, Colombia, in October 2014. In order to capture the genotypic variation of Phytophthora populations in Latin America, we developed a genotyping service to researches and companies of partner countries

of the network. We received samples from Chile, Brazil, Panama and Colombia from different Solanaceae hosts and analyzed them using a standardized 12 plex SSR genotyping (Li et al., 2013). The preliminary results had shown diversity of genotypes in Latin American Phytophthora populations. Each research group is analyzing in depth those preliminary results obtained to discuss them in the Third Workshop Tizon Latino 2018 to be held in Cusco, Peru, in the framework of World Potato Congress 2018 / ALAP 2018.

Based on our experience to adapt and develop new protocols, we suggest new genomic approaches to study Phytophthora populations.

TARGET SEQUENCING

Numerous papers report studies on specific regions, performed with Sanger sequencing. Currently, this robust technique is laborious and expensive to undertake epidemiological genomic studies that include numerous regions and isolates. Lange et al. (2014) proposed a protocol that allows the study of lots of regions in parallel on whole collections, using microfluidic units of Access Array (Fluidigm) technology that performs amplifications in sets of 48 samples per 48 regions amplified (2048) on a single chip, at a low cost per data point.
Amplicon sequencing is asayed in Illumina MiSeq system, obtaining read length to 2 × 250 base pairs.

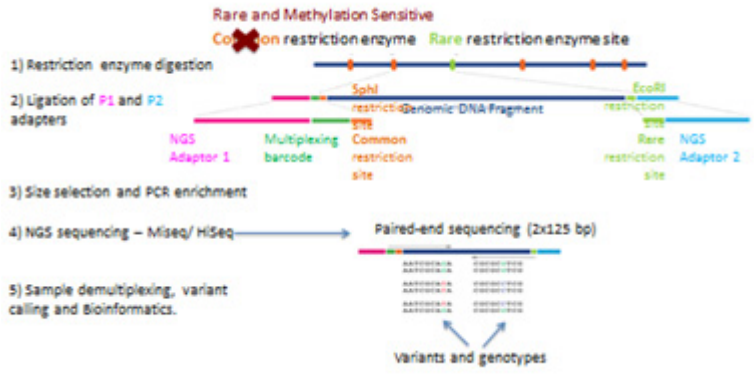


© Lange et al., 2014

DDRAD PROTOCOL – GBS

Different types of molecular markers are reported to date, and SNPs are currently the most widely used. Restriction enzyme genome-reduction methods, combined with Next Generation Sequencing (NGS), enable in a single assay to discover and genotype a large number of particular SNPs (in the order of thousands) of the study population. These methods are usually

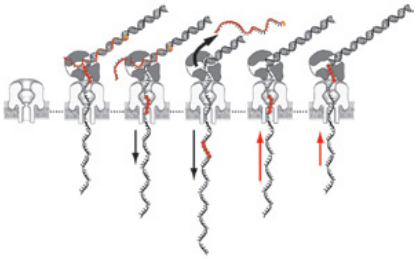
called GBS, and allow different population studies to be carried out at a lower cost than using microarrays and without the need for previous molecular information on the species (Davey et al., 2011). The double digest Restriction Associated DNA sequencing strategy (ddRADseq, Peterson et al., 2012), based on genome digestion with a double enzyme, combined with efficient prediction of the enzyme pair and size selection (selection of DNA fragments by size) optimal for the species of interest, is one of the most promising methodologies of GBS.
The development of the methodology of ddRADseq for *P. infestans* was performed in the Genomic Unit based on Aguirre et al protocol.



Aguirre et al., 2016

WHOLE GENOME SEQUENCING

P. infestans genome, was described by Haas et al. in 2009, this large and complex genome of 240 megabases (Mb) size results from a proliferation of repetitive DNA accounting for approximately 74% of the genome. It is a challenge for researchers to achieve protocols that allow obtaining of sequences at low costs and with results that allow the easy assembly of them.
First Draft Genome Sequence of the Pathogenic Fungus *Lomentospora prolificans* (formerly *Scedosporium prolificans*) was described recently. Sequencing and assembly of the fungus was performed using a combination of short, highly accurate Illumina reads and additional coverage in very long Oxford Nanopore reads (Luo et al., 2017).
These techniques are available in the genomic Unit and protocols can be assayed to obtained sequences of important isolates of *P. infestans* in Latin America.



The genomic approaches described in this work will be evaluated in the framework of different research projects of partner countries of Tizón Latino Network in order to improve understanding *P. infestans* populations in Latin America and to enable better late blight management. The newly genomic capacities of INTA are available for other research networks worldwide.

REFERENCES

Aguirre, N., Filippi, C., Zaina, G., Rivas, J., Acuña, C., Villalba, P., Garcia, M., Scaglione, D., Morgante, M., González, S., López, J., Marcucci, S. (2016). Development of a genotyping by sequencing strategy for assisted breeding of *Eucalyptus dunnii*. Technical Report · August 2016 DOI: 10.13140/RG.2.2.28273.07520

Carter, D.A., Archer, S.A., Buck, K.W., Shaw, D.S., Shattock, R.C. (1990). Restriction fragment polymorphisms of mitochondrial DNA of *Phytophthora infestans*. Mycol. Res. 94:1123–28.

Danies, G., Myers, K., Mideros, M., Restrepo, S., Martin, F.N., Cooke, D.E.L, Smart, C.D., Ristaino, J.B., Seaman, A.J., Gugino, B.K., Grünwald, N.J. & Fry, W.E. (2014). An ephemeral sexual population of *Phytophthora infestans* in the northeastern United States and Canada. PLOS ONE 9(12): e116354.

Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M. and Blaxter, M.L. (2011). Genome-wide genetic marker discovery and genotyping using Next-Generation Sequencing. Nature Reviews, Genetics, Volume 12, July 2011, 499 - 510.

Dong, S., Stam, R., Cano, L.M., Song, J., Sklenar, J., Yoshida, K., Bozkurt, T.O., Oliva, R., Liu, Z., Tian, M., Win, J., Banfield, M.J., Jones, A.M., van der Hoorn, R.A., Kamoun, S. (2014). Effector specialization in a lineage of the Irish potato famine pathogen. Science 343:552–55

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., Mitchell, S.E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLOS ONE 6:e19379.

Fry, W.E., Birch, P.R.J., Judelson, H.S., Grünwald, N.J., Danies, G., Everts, K.L., Gevens, A.J., Gugino, B.K., Johnson, D.A., Johnson, S.B., McGrath, M.T., Myers, K.L., Ristaino, J.B., Roberts, P.D., Secor, G., and Smart, C.D. (2015). Five reasons to consider *Phytophthora infestans* a reemerging pathogen. Phytopathology 105:966-981.

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

Goss, E.M., Tabima, J.F., Cooke, D.E.L., Restrepo, S., Fry, W.E., Forbes, G.A., Fieland, V.J., Cardenas, M. & Grünwald, N.J. (2014). The Irish potato famine pathogen *Phytophthora infestans* originated in central Mexico rather than the Andes. PNAS 111:8791–96.

Haas, B.J. et al*. (*2009). Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans.* Nature 461, 393-398 (17 September 2009) | doi:10.1038/nature08358.

Hansen, J.G. et al. (2009). The development and control of Late Blight *(Phytophthora infestans)* in Europe in 2007 and 2008*.* Eleventh EuroBlight Workshop Hamar - Norway. PPO Special Report 13 H.T.A.M. Schepers (editor):11-30.

Hansen, Z.R., Everts, K.L., Fry, W.E., Gevens, A.J., Grünwald, N.J., Gugino, B.K., Johnson, D.A., Johnson, S.B., Judelson, H.S., Knaus, B.J., McGrath, M.T., Myers, K.L, Ristaino, J.B., Roberts, P.D., Secor, G.A. & Smar, C.D. (2016). Genetic Variation within Clonal Lineages of *Phytophthora infestans* Revealed through Genotyping-By-Sequencing, and Implications for Late Blight Epidemiology. PloS one, 11(11), e0165690.

Haverkort, A.J., Struik, P.C., Visser, R.G.F. and Jacobsen, E. (2009). Applied Biotechnology to Combat Late Blight in Potato Caused by *Phytophthora infestans*. Potato Research 52:3, 249–264.

Kadish, D., Grinberger, M. & Cohen, Y. (1990). Fitness of metalaxyl-Sensitive and Metalaxyl-Resistant Isolates of *Phytophthora infestans* on Susceptible and Resistant Potato Cultivars. Phytopathology 80:200-205.

Lange, V., Böhme, I., Hofmann, J., Lang, K., Sauter, J., Schöne, B., Paul, P., Albrecht, V., Andreas, J.M., Baier, D.M., Nething, J., Ehninger, U., Schwarzelt, C., Pingel, J., Ehninger G. & Schmidt, A.H. (2014). Cost-efficient high-throughput HLA typing by MiSeq amplicon sequencing. BMC Genomics 201415:63 https://doi.org/10.1186/1471-2164-15-63

Li, Y., Cooke, D.E., Jacobsen, E., van der Lee, T. (2013). Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen *Phytophthora infestans*. J. Microbiol. Methods 92:316–22.

Lucca, M.F. & Rodriguez, J. Phytoalert: when less is more. Proceedings of the fifteenth EuroBlight Workshop. PPO-Special Report no. 17. December 2015. H.T.A.M. Schepers (editor) pp. 243-248.

Luo, R., Zimin, A., Workman, R., Fan, Y., Pertea, G., Grossman, N., Wear, M.P., Jia, B., Miller, H., Casadevall, A., Timp, W., Zhang, S.X., Salzberg, S.L. (2017). First Draft Genome Sequence of the Pathogenic Fungus *Lomentospora prolificans* (formerly *Scedosporium prolificans).* G3 (Bethesda). 2017 Sep 29. pii: g3.300107.2017. doi: 10.1534/g3.117.300107.

Mantecón, J.D. (1998). Potential yield losses caused by Late blight in Argentina during the last decade. Fungicide and Nematicide Test 53: 202-204.

Mantecón, J.D. (2000). Management of potato Late blight with several mancozeb formulations. Fungicide and Nematicide Test 55: 217-218.

Nielsen, B.J. (2014). Efficacy of fluazinam for control of potato late blight (*Phytophthora infestans*) in Danish field trials. Fourteenth EuroBlight Workshop Limassol – Cyprus. PPO Special Report 16, H.T.A.M. Schepers (editor): 113-116.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S. & Hoekstra, H.E. (2012). Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species PLoS ONE7(5): e37135.

Tooley, P.W., Fry, W.E. (1983). Genetic variation in *Phytophthora infestans* identified through isozyme analysis. Phytopathology 73:827