Late blight: pathogen variability and disease resistance breeding in Ecuador

Ricardo Delgado

Late blight: pathogen variability and disease resistance breeding in Ecuador

Ricardo Delgado

Thesis committee

Promotor
Prof. Dr. Richard G.F. Visser
Professor of Plant Breeding
Wageningen University & Research

Other members
Prof. Dr. Titti Mariani, Radboud University Nijmegen
Prof. Dr. Paul Struik, Wageningen University & Research
Prof. Dr. Anton Haverkort, Nigde University, Turkey
Dr. Conny Almekinders, Wageningen University & Research

This research was conducted under the auspices of the Graduate School of Experimental Plant Sciences.

Late blight: pathogen variability and disease resistance breeding in Ecuador

Ricardo Delgado

Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof.Dr A. P. J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Monday 21 October 2019
at 4 p.m. in the Aula.

Ricardo Delgado
Late blight: pathogen variability and disease resistance breeding in Ecuador
136 pages

PhD thesis, Wageningen University, Wageningen, The Netherlands (2019)
With references, with summaries in English and Spanish

ISBN: 978-94-6343-947-3
DOI: https://doi.org/10.18174/475569

Contents

	Page
Chapter 1 General Introduction	9
Chapter 2 Breeding for potato late blight resistance in Ecuador: INIAP's efforts	21
Chapter 3 Large sub-clonal variation in *Phytophthora infestans* populations associated to Ecuadorian potato landraces	35
Chapter 4 Exploring the reaction of Ecuadorian potato landraces to late blight	63
Chapter 5 Farmers perception of Ecuadorian potato landraces	75
Chapter 6 Construction of a linkage map using DArT markers and searching for QTLs for late blight resistance in a progeny of a cross between two Ecuadorian potato landraces	91
Chapter 7 Late blight response and yield characteristics of different potato varieties in two environments in Chimborazo province, Ecuador	103
Chapter 8 General Discussion	113
Summary	125
Resumen	127
Acknowledgements	129
About the author	130
List of publications	131
Education Statement	133

Dedication

To my wife Jennifer and my daughter Silvia Pilar

To my parents Julio César and Pilar

CHAPTER 1

General Introduction

Potato crop

The potato belongs to the Solanaceae family which was first domesticated in South America in the surroundings of Lake Titicaca which today is the border between Perú and Bolivia about 8000 years ago (De Jong, 2016). It allowed the development of societies across the Andes, including the Inca Empire by the time of the arrival of the Spanish conquestors. After this, potatoes were initially introduced to the Canary Islands and in Seville, Spain in the 1500s and, from there they were later distributed to the rest of the world (Brown & Henfling, 2014; Bentley, 2015, De Jong, 2016). Potato tubers are widely consumed due to their high carbohydrate and protein contents, additionally they are a valuable source of nutrients like Potasium, Calcium, Phosphorus, Copper, Magnesium and vitamins C and B (Camire et al, 2009; Stoorey 2007, Navarre et al, 2014).

Currently, the potato is the fifth most important crop in the world after, corn, wheat, rice and sugar cane with 376 million tons produced, it occupied the seventeenth place for cultivated area with 19 million ha in 2016 (http://www.fao.org/faostat/en/#home). It is cultivated around the world, with China, India, Rusia and Ukraine being the top four countries in area harvested and production, whereas The United States of America, New Zealand, Germany, Denmark and The Netherlands have the highest yields per ha (Table 1). In South America, the top producers are Peru, Brasil, Colombia and Argentina, meanwhile the highest yields are obtained in Brazil, Argentina, Chile and Uruguay (Table 2).

Potato in Ecuador

One of the most important crops in Ecuador is the potato, therefore it is an important part of the dietary consumption, especially in the highlands (Devaux *et al.*, 2010). The annual consumption per capita in the country is about 31.8 kg per year (Devaux *et al.*, 2010). It is cultivated in the Ecuadorian highlands from 2.000 to more than 3.600 meters above sea level (masl). Small scale farmers mainly cultivate it (around 45.000 of them) in an area of 29.635 hectares in 2016 (INEC, 2016) (Table 3). Most of the small farmers, about 88%, cultivate their potatoes in farms of no more than 20 ha (Rivadeneira, 2009). Even more, about 50% of the growers produce their potatoes in properties of less than 1 ha (Monteros-Guerrero, 2016).Potatoes are mainly produced in the provinces of Chimborazo (7.450 ha), Carchi (7.241 ha), Cotopaxi (3.909 ha), Pichincha (2.969 ha), Tungurahua (2.077 ha), and Cañar (1.995 ha) (Table 3 & Figure 1). In 2016, 422.589 tons were harvested (INEC, 2017). The main producing provinces are Carchi which represents 36.14 % of the total harvest, followed by Chimborazo (26.88%) and Cotopaxi (11.11 %) (Table 3). The national average yield was 12.71 t/ha in 2016, with the highest yields obtained in Carchi province (Table 3).

Ninety per cent of the potatoes are consumed fresh and the rest are used as chips and french fries (Devaux *et al.*, 2013). In the Northern provinces (Carchi and Imbabura) the growers prefer the Superchola variety which has a pink skin and yellow flesh. In the central provinces (Pichincha, Cotopaxi, Tungurahua, Chimborazo and Bolivar) the preferred variety is INIAP-Fripapa. In the southern provinces (Cañar, Azuay and Loja), Bolona variety, which is a landrace has the preference of the farmers; its tubers have a creamy coloured skin with creamy coloured flesh and a round shape (Devaux *et al.*, 2013) (Figure 2). According to Monteros-Guerrero (2016), Superchola variety (55%) is the most planted potato variety in the country followed by INIAP-Fripapa (5%) and in lower percentages several other clones.

Other particularities about the potato cultivation in Ecuador include that about 85% of the growers use their own seed obtained from the previous harvest and not from formal seed sources. Additionally, due to their small scale, most of them have difficulties with using technologies like irrigation, resulting in just 33% of the farmers with access to it (Monteros-Guerrero, 2016). Regarding the educational level of the farmers, 58% had elementary school partial or completed, 26% high school, 4% university and 7% no formal education (Flores et al, 2012). Aside from all this, there are biological stresses that affect the crop, reducing the yields and among them late blight had been consistently the most important one (Oyarzún et al, 2002; Devaux et al, 2010; Monteros-Guerrero, 2016; Navarrete et al, 2017).

Table 1. Potato statistics of main countries on area harvested (ha), production (t) and average yield (t/ha) in 2016.

Country	Area harvest (ha)	Country	Production (t)	Country	Average Yield (t/ha)
China	5812865	China	99065724	United States of America	49.02
India	2130000	India	43770000	New Zealand	48.99
Rusia	2030858	Rusia	31107797	Germany	44.42
Ukraine	1311600	Ukraine	21750290	Denmark	42.48
Bangladesh	475699	United States of America	19990950	Netherlands	42.00
United States of America	407810	Germany	10772100	Australia	40.41
Canada	342409	Bangladesh	9474099	Jordan	40.05
Nigeria	333100	Poland	8872445	Ireland	39.11
Poland	311620	France	6834680	France	39.01
Peru	310698	Netherlands	6534338	United Kingdom	38.65
World	19246462	World	376826967	World	19.58

FAOSTAT (http://www.fao.org/faostat/en/#home).

Table 2. Potato statistics of South American countries on area harvested (ha), production (t) and average yield (t/ha) in 2016.

Area	Area harvested (ha)	Production (t)	Yield (t/ha)
Argentina	59834	1750000	29.25
Bolivia	181708	1073744	5.91
Brazil	129842	3851396	29.66
Chile	53485	1166024	21.80
Colombia	121920	2354862	19.31
Ecuador	29635	422589	14.26
Paraguay	250	3625	14.50
Peru	310698	4400295	14.16
Uruguay	4424	89000	20.12
Venezuela	20202	391433	19.38

FAOSTAT (http://www.fao.org/faostat/en/#home).

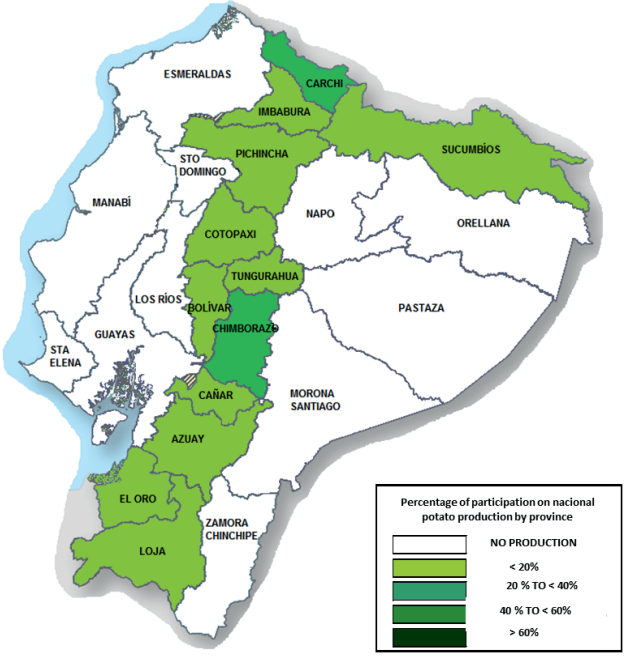


Figure 1. Potato production areas and percentage by province in Ecuador (INEC, 2017).

Table 3. Area harvested (ha) and pro-duction (tons) in the provinces where potatoes are cultivated in Ecuador.

Province	Area harvested (ha)	Percentage (%)	Production (t)	Percentage (%)	Average Yield (t/ha)
Azuay	1341	4.53	7298	1.73	5.44
Bolívar	1611	5.44	12262	2.90	7.61
Cañar	1995	6.73	8461	2.00	4.24
Carchi	7241	24.44	152742	36.14	21.09
Cotopaxi	3909	13.19	46929	11.11	15.25
Chimborazo	7450	25.14	113588	26.88	12.01
Imbabura	923	3.11	9625	2.28	10.43
Loja	32	0.11	134	0.03	14.21
Pichincha	2969	10.02	42203	9.99	13.12
Tungurahua	2077	7.01	27257	6.45	18.69
El Oro	10	0.03	32	0.01	3.18
Sucumbíos	75	0.25	2057	0.49	27.31
Total	29635	100	422589	100	-
Average	-	-	-	-	12.71

/Adapted from data from INEC (2017).

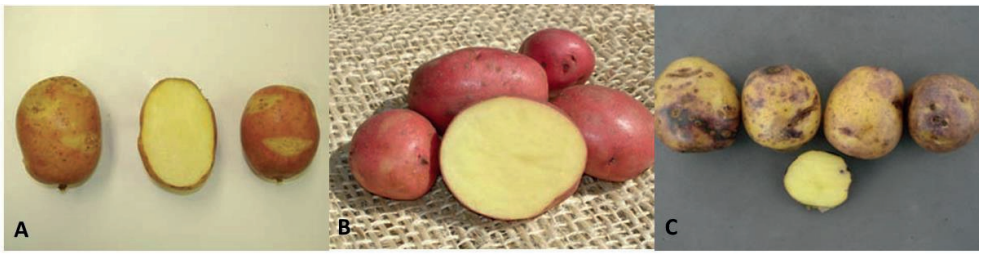


Figure 2. Tubers from Superchola (A), INIAP-Fripapa (B) and Bolona (C) potato varieties (Pumisacho & Velazquez, 2009).

Late Blight around the world and in Ecuador

Late blight, called in Ecuador ‘tizón tardío’ in Spanish or ‘lancha’ in kichwa, is caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. This is the main disease of potatoes worldwide (Thurston & Schultz, 1981). It was the cause of the known ‘Irish famine’ which caused the death of a million people and the immigration of another million in the 1840s (Nowicki et al, 2012; De Jong, 2016). The disease is still a matter of concern worldwide due

to the epidemics that reduce the yields and incomes among others because of the increase of the production costs in order to control this problem (Fry, 2008). The European Union has estimated a loss of 1 billion euros annually due to the disease (Haverkort et al, 2008). Also in the United States, the costs for the fungicides applied for the control of late blight have been calculated to be around 300 to 500 dollars per hectare (Johnson et al, 2000; Guenthner et al, 2001).

In Ecuador, late blight is the main constraint of this crop in the country (Oyarzún *et al.*, 2002). The first time the pathogen was described in Ecuador it infected the fruit bearing crops, sweet cucumber (*Solanum muricatum*) and tzimbalo (*S. caripense*) (Lagerheim, 1890). Years later, it was observed causing blight in potatoes (Pachano, 1918). Epidemics caused by this pathogen led to losses than can range from 28 to 100 % of the yield varying due to host resistance and weather conditions (Morales *et al.*, 1995).

The pathogen can infect leaves, stems, petioles and tubers (Oyarzún *et al.*, 2002). Leaf symptoms consist of brown spots of necrotic tissue often surrounded by a pale green to yellow halo (Figure 3). Lesions can expand and coalesce with each other, killing the leaflets, leaves and eventually the whole plant (Figure 3) (Thurston & Schultz, 1981). When high moist weather conditions occur sporangia are formed in the abaxial leaf surface (Figure 3) (Thurston & Schultz, 1981). Stem lesions can also occur as dark brown spots which can break by wind or other movements (Figure 3) (Perez & Forbes, 2010). The foliage damage affects the photosynthesis reducing the number and weight of the tubers. The damage can be so extensive that the whole plant is destroyed (Thurston & Schultz, 1981).

The life cycle of the pathogen consists of asexual reproduction through sporangia which germinate directly producing mycelia or by realizing motile zoospores (Figure 4 & Figure 5). These spores later lose their flagella and germinate causing infection through a germ tube. Until this moment, only the asexual cycle of the pathogen seems to exist in Ecuador (Oliva *et al.*, 2002). The disease cycle starts from the sporangia grown on the surface of the infected tissue which has gotten necrotic. These can be dispersed by rain and wind. Once landed on susceptible tissue, sporangia can germinate directly producing mycelia which infect the plant or by realizing zoospores. The pathogen can survive in infected tubers, debris and volunteer plants. Infection of tubers can occur but is rarely observed in Ecuador (Oyarzún *et al.*, 2005, Kromman *et al.,* 2008b), possibly due to suppressiveness of the soil, either by biological and/or soil factors (Oyarzún *et al.,*2011, Orquera-Tornakian *et al*, 2018). Late blight epidemics could start soon after the emergence through infected sprouts from zoospores or sporangia from infected potato plants in the field (Kromman *et al.,* 2008b).

Two clonal lineages, EC-1 and US-1, both of the A1 mating type have been identified in Ecuador (Forbes *et al.*, 1997). The EC-1 lineage, is predominant in the potatoes and US-1 in tomatoes (Forbes *et al.*, 1997; Oyarzún *et al.*, 1998). These populations have been changing through the years, from a predominance of avirulent isolates (INIAP, 1976) to complex races (Forbes *et al.*, 1997, Tello, 2008). The EC-1 lineage was found to be more aggressive than the US-1 (Ordoñez *et al.*, 1998).

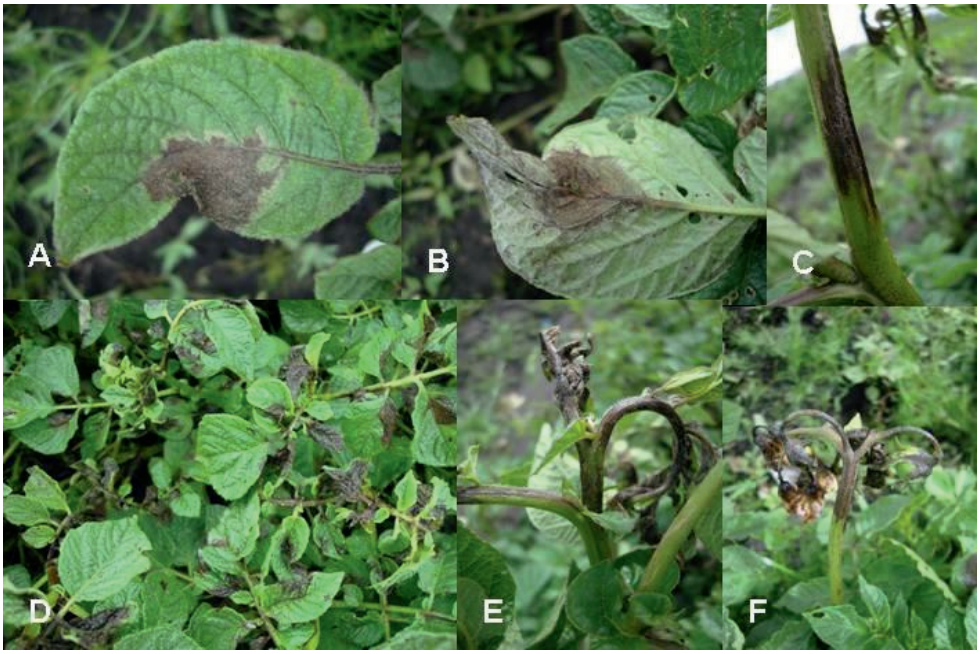


Figure 3. Symptoms of late blight in potato: Lesion on a potato leaflet caused by *P. infestans* surrounded with a yellow halo (A); Sporulation on the abaxial surface of leaflet (B); Stem lesion (C); Necrotic lesions on foliage (D); Blight lesions on the apex of the plant (E); Blight of the inflorescence (F).

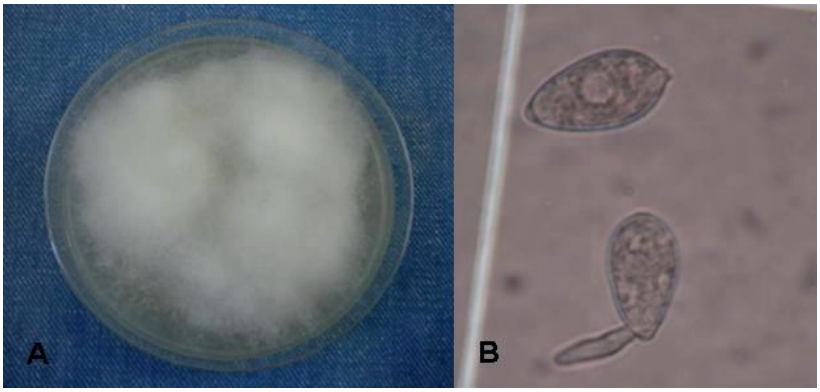


Figure 4. Colony of *P. infestans* growing in Rye Agar media (A); Sporangia of *P. infestans* (B).

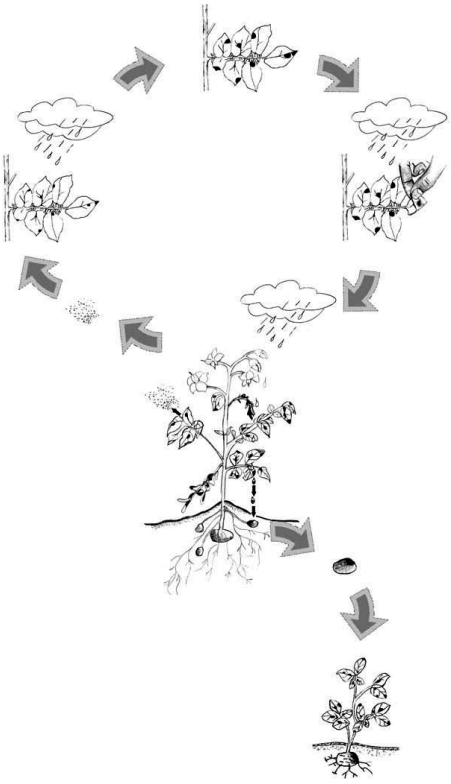


Figure 5. Disease cycle of potato late blight in Ecuador (Cáceres *et al.*, 2007).

Disease management

For the management of the disease, two alternatives have been applied, chemical control by the application of fungicides and the use of resistant varieties. The chemical control is used in the commercial crops in order to prevent epidemics. It has been ongoing since the first description of this disease in the country when Pachano (1918) suggested the use of the Bordeaux mixture in potatoes. Several types of fungicides have been recommended through the years in order to minimize the losses due to late blight, including those with contact or systemic mode of action (Vega, 1975, Muñoz & Cruz, 1984, Padilla, 1991, Oyarzún *et al.*, 2002, Taipe *et al.*, 2002, Mideros & Forbes, 2003, Caceres *et al.*, 2007, Kromman *et al.*, 2008a). It is recommended to start applying fungicides when severity reaches 0.5% of late blight or even as preventive measure soon after emergence of the plants (Oyarzún *et al.*, 2002). This control measure increases the costs due to repeated sprays over the plants; they can be as high as 14 times in a crop cycle which have been observed in Carchi province in the susceptible variety Superchola (Yánez *et al.*, 2008). It has been estimated that the cost of late blight control can be as high as 20% of the total income (Oyarzún *et al.*, 2001). Another problem due to the high use of the fungicides is that they can affect the health of the farmers

as reported by Crissman *et al.,* (1994), Cole *et al.,* (1997) and Yanggen *et al.,* (2003) who reported cases with symptoms of skin irritation.

The other control method available is the planting of late blight resistant varieties. This is thought to be the most suitable control because it is supposed to be easy to adopt. Despite this, it has been observed in different countries that late blight susceptible varieties are preferred by farmers due to their quality and consumer preferences, like is the case for the Granola and Superchola varieties in Indonesia and Ecuador, respectively (Forbes, 2012). Other advantages of the use of resistant varieties are that they are friendly for the environment and safe for farmers.

The main objective of breeding potatoes in Ecuador is obtaining improved varieties with valuable traits including late blight resistance. For this purpose different sources of genes are used, among them potato landraces which include species like *S. phureja*, *S. andigena* and *S. chaucha* (Monteros-Altamirano, 2011). Additionally introduced germplasm from other locations is used for obtaining improved varieties. The breeding process until the release of a new variety takes many years (10 -15) (Cuesta, 2011; Cuesta et al, 2015). The obtaining of a new potato variety has to include several traits apart from late blight resistance, such as earliness and nutritional contents among others. Diverse factors may affect the breeding for late blight resistance, such as pathogen population and environment. In the case of pathogen population, it represents a complication for the breeder since it has been observed that late blight resistant varieties, were quickly overcome by the pathogen and thus the varieties status changed from resistant to susceptible relatively soon after they were released, as observed with Arka (Tanzania), Capiro (Colombia), INIAP-Cecilia, INIAP-Gabriela, Superchola (Ecuador), Canchan and Amarilis (Perú) (Forbes 2012). This leads to the constant need for new sources of resistance to be included in the breeding plan.

The development of the technologies for the potato crop in Ecuador, which includes improved varieties, integrated pest and crop management are the responsibility of the National Agricultural Institute of Ecuador (Instituto Nacional de Investigaciones Agropecuarias – INIAP), with emphasis on applied reseach.

Scope of this thesis

Ecuador has as a valuable resource in the potato landraces which have been grown for many decades by the farmers. They may be a source of quantitative resistance to late blight since they have survived the epidemics of late blight induced by a new aggressive population in the country. The objective of this thesis is to characterize the resistance of Ecuadorian potato varieties, understand why a clonal population of *P. infestans* makes it difficult to obtain durable resistance, and attempt to identify QTLs associated to the resistance against this important and devastating disease.

In chapter 2, the potato breeding work performed in Ecuador is presented and analyzed. Methodologies, germplasm utilized, and main achievements are presented. Perspectives for future development are discussed.

In chapter 3, the diversity of the populations of *P. infestans* associated to potato landraces is studied. The pathogenic diversity in the country is analyzed and the genetic variability is evidenced.

In chapter 4, a screening of late blight resistance is performed with a group of selected landraces, not reported elsewhere, in Carchi province with the aim to characterize their reaction against the disease and identify the possibilities of incorporating some of them as new parental clones for breeding.

In chapter 5, an evaluation of late blight field resistance among a selected group of landraces, collected recently, is performed and an analysis of the perception of this disease by small farmers is made. Their value for breeding purposes and reasons for their use and existence until the present days are discussed.

In chapter 6, the behavior of a group of selected potato varieties is studied in different sites in Chimborazo province. The genotype x environment interaction is analyzed and discussed.

In chapter 7, a QTL analysis is performed in a segregating population of a cross between two *S. phureja* landraces which were shown to carry some level of quantitative resistance against late blight in an attempt to see whether QTL could be identified and if yes whether these could be combined to obtain a higher level of resistance.

In chapter 8, a discussion on the main findings of this thesis is presented. Recommendations for future research are made.

Literature cited

Andrade, H., Bastidas, O., Sherwood, S. 2002. La papa en Ecuador. In Pumisacho M. & Sherwood S. (Eds.). El cultivo de la papa en el Ecuador. Quito, Ecuador: INIAP – CIP. Pp. 21-32.

Bentley, J.W. 2015. World history of potato. Revista Latinoamericana de la Papa 19, 2, 76-81.

Brown C.R., Henfling, J.W. 2014. A history on potato. In Navarre R, Pavek M.J. (eds.) The potato: botany, production and uses. Wallingford, UK: CABI. Pp. 1-11.

Cáceres, P.A., Pumisacho, M., Forbes, G.A., Andrade-Piedra, J.L. 2007. Guía para facilitar el aprendizaje sobre control de tizón tardío de la papa. Centro Internacional de la Papa (CIP), Instituto Nacional Autónomo de

Investigaciones Agropecuarias del Ecuador (INIAP), Secretaría Nacional de Ciencia y Tecnología del Ecuador (SENACYT). Quito, Ecuador. 142 páginas.

Camire, M., Kubow, S., Donnelly, D. 2009. Potatoes and Human Health. Critical Reviews in Food Science and Nutrition 49:823-840.

Crissman, C.C., Cole, D.C., Carpio, F. 1994. Pesticide use and farm worker health in Ecuadorian potato production. American Journal of Agricultural Economics 76 (3): 593-597.

Cole, D.C., Carpio, F., Math, J.J.M., León, N. 1997. Dermatitis in Ecuadorian farm workers. Contact dermatitis 37, 1-8.

Cuesta, X. 2011. Recursos genéticos de la papa y fitomejoramiento. In I Expo Congreso Nacional de Papa (Expopapa). 25-27 de noviembre de 2010 Ibarra, Ecuador.

Cuesta, X., Rivadeneira, J., Monteros, C. 2015. Mejoramiento genético de papa: Conceptos, procedimentos, metodologías y protocolos. Quito, Ecuaodr: Instituto Nacional de Investigaciones Agropecuarias. 62 p.

De Jong, H. 2016. Impact of the potato on society. American Journal of Potato Research 93 (5), 415-429. DOI 10.1007/s12230-016-9529-1

Devaux A, Ordinola M, Hibon A., Flores R.. 2010. El sector papa en la región andina: Diagnóstico y elementos para una visión estratégica (Bolivia, Ecuador y Perú). Lima, Perú: Centro Internacional de la Papa – CIP.

Flores R, Naranjo H, Galarraga J, Sanchez MP, Viteri S. 2012. Estudio de la demanda de semilla de papa de calidad em Ecuador. Quito, Ecuador: OFIAGRO-Centro Internacional de la Papa. 246 p.

Forbes GA. 2012. Using Host Resistance to Manage Potato Late Blight with Particular Reference to Developing Countries. Potato Research (2012) 55:205–216 DOI 10.1007/s11540-012-9222-9

Forbes GA, Escobar XC, Ayala CC, Revelo J, Ordoñez ME, Fry BA, Doucett K, Fry WE, 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. *Phytopathology* 87, 375-380.

Guenthner, J.F., Michael, K.C., Nolte, P. 2001. The economic impact of potato late blight on US growers. Potato Research 44: 121. https://doi.org/10.1007/BF02410098

Haverkort AJ, PM Boonekamp, R Hutten, E Jacobsen, LAP Lotz, GJT Kessel, JH Vossen & RGF Visser (2016). Durable late blight resistance in potato through dynamic varieties obtained by cisgenesis: scientific and societal advances in the DuRPh project. Potato Research 59:35-66.

INEC (Instituto Nacional de Estadísticas y Censos), 2017. Encuesta de superficie y producción agropecuaria contínua 2016. Quito, Ecuador: INEC.

INIAP (Instituto Nacional de Investigaciones Agropecuárias), 1976. *Informe Anual de Actividades*. Departamento de Fitopatología. Quito, Ecuador: INIAP.

Johnson, D. A., Cummings, T. F., and Hamm, P. B. 2000. Cost of fungicides used to manage potato late blight in the Columbia Basin: 1996 to 1998. Plant Disease 84:399-402.

Kromann P, Leon D, Taipe A, Andrade-Piedra JL, Forbes GA. 2008a. Comparison of two strategies for use of translaminar and contact fungicide in the control of potato late blight in the highland tropics of Ecuador. Crop Protection 27, 1098–1104.

Kromann, P., Taipe, A., Andrade-Piedra, J. L., Munk, L., Forbes, G. A. 2008b. Preemergence infection of potato sprouts by *Phytophthora infestans* in the highland tropics of Ecuador. Plant Dis. 92:569-574.

Lagerheim, G. 1890. La enfermedad de los pepinos, su causa y su curación. Revista Ecuatoriana 2: 484-488.

Mideros S., Forbes GA. 2003. Evaluation of chemical and biological fungicides for control of potato late blight in Ecuador, 2002. Fungicide and nematicide test 58: V067.

Monteros-Altamirano, A. 2011. Potato landraces: description and dynamics in three areas of Ecuador. PhD. Thesis. Wageningen, The Netherlands, Wageningen University.

Monteros-Guerrero, A. 2016. Rendimientos de papa en el Ecuador. Primer ciclo 2016. Quito: Ministerio de Agricultura, Ganadería y Pesca. 10 p. http://sipa.agricultura.gob.ec/descargas/estudios/rendimientos/papa/rendimiento_papa_2016.pdf

Morales, R.; Revelo, J.; Suárez, G.; Forbes, G. Muñoz, F. 1995. Relación entre el ataque de *Phytophthora infestans* y la producción en el cultivo de papa (*Solanum tuberosum*). In Memorias XVII Reunión de la Asociación Latinoamericana de la Papa (ALAP). Mérida, Venezuela. Pp. 73-74.

Muñoz F, Cruz L. 1984. Manual del cultivo de la papa. Quito, Ecuador: INIAP, Estación Experimental Santa Catalina.

Navarre, D., Goyer, A., Payyavula, R., Hellmann, H. 2014. Nutritional characteristics of potatoes. In Navarre R, Pavek M.J. (eds.) The potato: botany, production and uses. Wallingford, UK: CABI. Pp. 310-314.

Rivadeneira, J. 2009. El cultivo de papa y su participación en la economía ecuatoriana. Revista Batata Show 24, 35-41.

Pachano, A. 1918. Dos enfermedades de las papas. Quinta Normal Estación Experimental Circular 7: 1-11.

Padilla W. 1991. Evaluación del fungicida Patafol (Ofurace + Mancozeb) en el control de la lancha *Phytophthora infestans* en el cultivo de papa. In Memorias V Seminario Nacional de Sanidad Vegetal. Cuenca, Ecuador, Junio 7 – 9, 1988. Cuenca, Ecuador: Universidad de Cuenca. Pp. 100-101.

Perez W, Forbes G. 2010. Potato lae blight; Technical Manual. Lima, Peru: International Potato Center – CIP.

Pumisacho, M. & Velázquez, J. 2009. Manual del cultivo de papa para pequeños productores. Quito, Ecuador: INIAP-COSUDE.

Oliva, R. F., Erselius, L. J., Adler, N. E., Forbes, G. A. 2002. Potential of sexual reproduction among host-adapted populations of *Phytophthora infestans sensu lato* in Ecuador. Plant Pathology, 51: 710–719.

Orquera-Tornakian, G. , Díaz, C. I., Mogrovejo, D. C., Villamarín, D. J., Jarrín, F. , Ponce, L. K., Oliva, R. , Gia, J. , Forbes, G. A., Andrade-Piedra, J. , Flores, F. J., Garzon, C. D., Molineros, J. , Koch, A., Benítez, M. S. (2018), Characterization of tuber blight-suppressive soils from four provinces of the Ecuadorean Andes. Plant Pathol, 67: 1562-1573. doi:10.1111/ppa.12872

Oyarzún PJ, Garzón CD, León D, Andrade I, Forbes GA. 2005. Incidence of Potato Tuber Blight in Ecuador. Amer J of Potato Res 82:117-122.

Oyarzún PJ, Pozo A, Ordoñez ME, Doucett K, Forbes GA, 1998. Host specificity of *Phytophthora infestans* on tomato and potato in Ecuador. *Phytopathology* 88, 265-271.

Oyarzún PJ, Taipe JA, Forbes GA. 2001. *Phytophthora infestans* activity and particuliarities in Ecuador: Country Profile. Pages in: Proceedings of the International Workshop on Complementing Resistance to Late Blight (Phytophthora infestans) in the Andes. February 13-16, 2001, Cochabamba, Bolivia. GILB Latin American Workshops 1. E.N. Fernández-Northcote, ed. International Potato Center, Lima, Peru.

Oyarzún P, Gallegos P, Asaquibay C, Forbes G, Ochoa J, Paucar B, Prado M, Revelo J, Sherwood S, Yumisaca F. 2002. Manejo integrado de plagas y enfermedades. In: Pumisacho M, Sherwood S, eds. *El Cultivo de la Papa en Ecuador*. Quito, Ecuador: INIAP/CIP, 85–169.

Oyarzún, PJ, Krijger, AK, Garzón, CD, Leon D, Kromann P, Yuen, JE, Forbes GA. 2011. Evaluation of host susceptibility, pathogen aggressiveness and sporangial survival in soil as factors affecting incidence of potato tuber infection by *Phytophthora infestans* in Ecuador. *Tropical Plant Pathology*, vol. 36, 3, 141-149.

Storey, M. 2007. The harvested crop. In D. Vreugdenhil, et al. (Eds.), Potato Biology and Biotechnology, Elsevier Science B.V., Amsterdam. pp. 441-470.

Taipe A, Oyarzún P, Forbes G. 2002. Evaluación de nuevos fungicidas y productos alternativos para el control de "tizón tardío" (*Phytophthora infestans*) de la papa en el Ecuador. In Memorias XX Congreso de la Asociación Latinoamericana de la Papa. Junio 3 – 7, 2002. Quito, Ecuador: INIAP. P. 52.

Tello C, 2008. *Identificación de aspectos epidemiológicos relacionados con la expresión de resistencia de la papa (Solanum tuberosum) para las poblaciones de Phytophthora infestans predominantes en tres localidades de la sierra ecuatoriana.* Quito, Ecuador: Universidad Central del Ecuador, BSc Thesis.

Thurston H.D., Schultz, O. 1981. Late blight. In Hooker, W.J. (ed.) Compendium of potato diseases. St. Paul, U.S.: American Phytopathological Society, 40 – 42.

Vega JG. 1975. Combata la lancha de las papas usando fungicidas. Boletín divulgativo No. 76. Quito, Ecuador: INIAP Estación Experimental Santa Catalina.

Yánez, E., Espinoza, P., Sevillano, C., Yumisaca, F., Guerrero, D., Cuesta. X. 2008. Estudio de línea base de variedades de papa en comunidades de la provincia de Carchi, Chimborazo y parroquia Quero en Ecuador. In XXIII Congreso de la Asociación Latinoamericana de la Papa (ALAP). Pp.441-442.

Yanggen D, Crissman C, Espinosa P (eds). 2003. Los Plaguicidas: Impactos en producción, salud y medio ambiente en Carchi, Ecuador. Quito, Ecuador: CIP/INIAP.

Chapter 2

Breeding for potato late blight resistance in Ecuador: The National Agricultural Research Institute's INIAP's efforts

Ricardo A. Delgado[1,2,3] , Richard G.F. Visser[2]

[1] Instituto Nacional Autónomo de Investigaciones Agropecuárias (INIAP), Estación Experimental Santa Catalina, Panamericana Sur km 1, Quito, Ecuador.
[2] Plant Breeding, Wageningen University and Research, P.O. Box 386, 6700 AJ, Wageningen, The Netherlands.
[3] Graduate school Experimental Plant Sciences, Wageningen University

Abstract

Potato is one of the most important food crops in Ecuador. Late blight is the main disease that affects potatoes in the country. Breeding efforts for obtaining improved potato varieties have been carried out by the Instituto Nacional Autónomo de Investigaciones Agropecuárias (INIAP) soon after its creation in the late 1950ties. The objective of this assay is to review the breeding for potato late blight resistance in Ecuador carried out by the INIAP, the strategies followed and results achieved in the last 60 years.

Keywords: *Phytopthora infestans*, *Solanaceae*, Oomycete

Introduction

Potato is one of the most important staple crops in Ecuador. It is an important part of the diet of all people in the country, especially in the highlands (Devaux *et al.*, 2010). Potatoes are cultivated in the Ecuadorian highlands (Figure 1), mostly by small scale farmers (Andrade *et al.*, 2002). Potatoes are grown all year around mainly based on the seasonal rainfall and the access to irrigation. The plantings are made from May to June and October to December. The average national yield was in 2016, 12.21 t/ha (INEC, 2017), which is lower than those obtained in Colombia (19.31 t/ha) and Perú (14.16 t/ha) (http://www.fao.org/faostat/en/#home). This may be explained by the fact that most of the farmers use seed tubers selected from the harvested production (Flores *et al.*, 2013) , which is true for about 85% of the seeds used in the country (Monteros-Guerrero, 2016). This situation leads to a diminished sanitary quality of the seeds necessary to ensure good yields (Navarrete et al, 2017).

Among the factors that limit the yield, there is late blight which is caused by the oomycete *Phytophthora infestans* (Mont.) de Bary and it is the main disease of potatoes in the country (Oyarzún *et al.*, 2002). The pathogen was first reported in Ecuador in 1890 infecting *Solanum muricatum* and *S. caripense*, probably the first report of a plant disease in the country (Lagerheim, 1890). Later, it was confirmed to cause blight in potatoes as well (Pachano, 1918). It can induce severe losses depending on host susceptibility and weather conditions that might favor the epidemics (Morales *et al.*, 1995). For the management of the disease, two alternatives have been applied; chemical control by the application of fungicides and the use of resistant varieties. Chemical control is used in the crop in order to prevent epidemics in commercial fields (Oyarzún *et al.*, 2002). It may require repeated sprays over the plants; they can be as high as 14 times as has been observed in Carchi province (Yánez *et al.*, 2008). Another problem due to the high use of the fungicides is that they affect the health of the farmers as reported by Crissman *et al.*, (1994). The most suitable control measure is the use of resistant varieties because this does not require the use of fungicides anymore and it is supposed to be easy to adopt. The objective of this essay is to review the breeding for potato late blight resistance in Ecuador in the last 60 years, the strategies followed, results achieved and future perspectives.

Potato Breeding in Ecuador

The breeding efforts in the country, started in Ambato in the 1910's with the trials set by the Quinta Normal de Agricultura- an agricultural school- where native potatoes and newly introduced varieties were evaluated for late blight resistance (Pachano, 1918). Some years later (in the 1930's), crosses among *S. andigena* local varieties were performed by Manuel Bastidas, a private breeder from the Carchi province producing some improved varieties which were planted in that location (Estrada, 2000). The work of this breeder was followed by his son Germán Bastidas who released some varieties including Superchola (Bastidas, 1991), which is still one of the most cultivated commercial varieties in Ecuador (Pumisacho & Velasquez, 2009; Monteros-Guerrero, 2016).

The National Agricultural Research Institute of Ecuador (INIAP) was created in 1959. In 1961, a collection of three hundred clones was received from the Universidad Central del Ecuador and transferred to the Potato Program. This germplasm was named as Colección Ecuatoriana de la Papa- CEP (Ecuadorian Potato Collection) starting in this way the potato breeding activities of INIAP (Albornoz & Morillo, 1968).

Breeding Objectives of INIAP's Potato Program

Nowadays, the potato program has as breeding objectives to obtain improved varieties adapted to the conditions of the country, with the following attributes: high yielding (>30 t/ha), earliness (growth cycle shorter than 150 days), resistant against main biotic stresses (*Phytophthora infestans*, *Rhizoctonia solani*, *Pectobacterium* sp., *Globodera* sp. and viruses), resistant against abiotic stresses (drought, high or low temperatures, poor soils), suitable for fresh consumption (round shape, high contents of iron and zinc, and antioxidants) or industrial purposes like chips and fries (round to oblong shape, high dry matter content, etc) (Figure 3) (Cuesta, 2011; Cuesta et al, 2015). Breeding of varieties includes different

approaches like clonal selection and hybridization which can take more than 10 years (Figure 2, 4).

Breeding strategies

Initially the breeding efforts consisted of the selection of clones from local germplasm and introduced from other origins like Colombia. Hence, in 1965, INIAP-Santa Catalina, a late blight resistant variety was released. This was the first variety offered by INIAP (Albornoz & Ortuño, 1968).

In collaboration with the International Potato Center (Centro Internacional de la Papa -CIP) in Peru, advanced clones were introduced and varieties were selected. Most of them had monogenic resistance which was effective when the varieties were released, but quickly were overcome by the pathogen (Revelo *et al.*, 1997). This was due to a shift of the dominant population of *P. infestans*, which was more aggressive (Forbes *et al.*, 1997) and has a high genetic diversity (Chapter 3; Delgado *et al.*, 2013).

During part of the 1990's a strategy for breeding based on quantitative resistance against late blight was implemented. The aim was to obtain varieties with durable resistance. It consisted of the screening of clones from the CEP and other origins for late blight resistance at field level. Additionally, the clones were tested using a detached leaf assay in the laboratory with a complex race and a race 0 of *P. infestans*. The purpose was to select progenitors that had resistance but no major genes, thus selecting for quantitative or so called field resistance against late blight (Cuesta *et al.*, 1999). To this scheme a recurrent selection procedure was added so that after some cycles of field trials advanced clones were selected as progenitors and introduced into the breeding process by crossing them with other parental lines and starting over the selection on the resulting materials.

A new approach was introduced in the Potato Program of INIAP, the so called Participatory breeding (PB). The purpose was to improve the breeding process by the inclusion of the criteria of the farmers, consumers, and the industry. All these had the goal to improve the acceptance and adoption of the new varieties. The PB started in 1992 as part of the breeding scheme in INIAP's Potato Program (Capelo, 1998). The procedure included the evaluation of different traits by selected groups of potential users of the varieties, such as farmers, consumers, industrial representatives among other actors involved in the potato chain. The traits under evaluation were late blight resistance, yield and organoleptic characteristics (Capelo, 1998, Cardenas *et al.*, 1998). Varieties such as INIAP-Raymipapa and INIAP-Suprema were selected using this approach (Cuesta *et al.*, 2000). PB is nowadays a routine part of the breeding process carried out by INIAP's Potato Program.

Other strategies have been explored over the years as well, like mutation breeding, with some results. Chacon & Forbes (2000) from CIP, irradiated plants of the varieties INIAP-Esperanza and INIAP-Cecilia. They observed that some of the plants were more resistant to late blight than the non-irradiated controls. They did not find immunity in the individuals so it was concluded that the irradiation induced variation in quantitative resistance. Years later, Lopez & Yánez (2010) from INIAP, tried to induce mutations by irradiation in the late blight susceptible variety Superchola. Eighteen mutants performed better than untreated Superchola,

but were not as resistant as INIAP-Santa Catalina, the resistant control. Despite these experiences, no improved varieties have been developed by this technique until this moment.

Potato landraces were part of the beginning of the Potato Breeding Program as part of the CEP (Albornoz & Ortuño, 1968, Sola, 1986, Andrade *et al.*, 1994, Andrade, 2002). These potatoes are the result of selection and conservation carried out by small scale farmers in the highlands over generations (Cuesta *et al.*, 2005). They selected tubers from each crop cycle as seeds for new plantings. They constitute a potential source of genetic variation for breeding purposes.
Despite the fact that most of them are susceptible to late blight, some accessions have quantitative resistance (Cañizares & Forbes, 1995; Revelo *et al.*, 1997; Garofalo *et al.*, 2005; Delgado & Vosman, 2009, Monteros-Altamirano 2011). They could also be a source of genes to improve nutritional contents like carotenoids and polyphenols as reported by Cuesta-Subia *et al.*, (2012). Efforts have been carried out in order to preserve the landraces, characterize and use them for breeding purposes (Andrade *et al.*, 1994, Revelo *et al.*, 1997, Monteros-Altamirano, 2011). These attempts have been conducted since the beginning of the breeding program because the preference of consumption in the local markets tended to the old traditional varieties (Reinoso, 1994a & 1994b). The importance of landraces is illustrated in the genealogy of most of the varieties developed by INIAP (Table 1). An example of the variability on shape and color of Ecuadorian potato landraces can be observed in Figure 3.

Other approaches for variety improvement include the introduction of genes from wild species like *S. pausissectum*, *S. bulbocastanum* and *S. okadae* into potato in order to obtain varieties with late blight resistance. From crosses performed between different varieties (like INIAP-Gabriela and Superchola) and hybrids of varieties with landraces such as Yema de Huevo (*S. phureja*) x *S. pausissectum*, the INIAP-Natividad and INIAP-Estela varieties were obtained (Cuesta *et al.*, 2007a, Cuesta *et al.*, 2007b). In the case of *S. bulbocastanum* and *S. okadae*, clones were obtained from protoplast fusion with *S. tuberosum* performed by the University of Tubingen in Germany. These materials were introduced to Ecuador carrying genes of these wild *Solanum* species (Schilde, 2003). Field evaluation of the clones allowed the identification of three late blight resistant clones (Espinoza & Andrade, 2007).

Discussion

To date, twenty-one potato varieties have been released by INIAP over the last sixty years (Table 1). However, the varieties developed by INIAP have only been very limited adopted by most of the farmers. The main commercial varieties planted in the country are INIAP-Fripapa and Superchola (Pumisacho & Velasquez, 2009). Despite its high susceptibility to late blight, Superchola is still predominant among the improved varieties planted in commercial farms. One explanation for this apparent contradiction is that the characteristics that are demanded in the markets are outstanding in this variety. These are pink skin, superficial eyes and yellow flesh (Andrade *et al.*, 1997). A study carried out in the early 1990s in the markets of Quito, Guayaquil and Cuenca –the main cities of Ecuador-, showed that consumer preferences tended to Superchola and local varieties (Reinoso, 1994a & 1994b). Surveys performed in the restaurants in Quito showed that Superchola is the most demanded potato variety in the city for processing purposes (Mayorga, 2006; Alcozer, 2006). Additionally, according to Sherwood (2009) consumers in the fresh market tend to buy a type

of potato that fits with Superchola characteristics, so all new varieties should be similar to it (Table 2). Another example on the difficulties for the adoption of the improved varieties is the case of INIAP-Estela which is resistant to late blight, high yielding, drought resistant and selected according preferences of the farmers. Unfortunately, it didn't match with the preferences of the traders and consumers who did not like the purple skin and the light cream flesh color, so the farmers stopped planting it since there were no buyers. Hence, it is necessary to include in the participatory breeding the traders and consumers in order to select potato varieties that filll the requirements of all the stakeholders (X. Cuesta, INIAP, personal comm.).

The population of the pathogen adds an additional difficulty due to its complexity and even genetic diversity as reported by Forbes *et al.* (2001) and Delgado *et al.* (2013). This implies the need of sources of resistance genes which must be effective against complex races of the pathogen.

It is also necessary to promote the use of a formal seed distribution system since nowadays most of the farmers use the tubers from their own harvests (Monteros-Guerrero, 2016), thus making it difficult to adopt the improved potato varieties.

The breeding process has to include a lot of traits which make the process difficult and requires years until new varieties can be released. Other alternatives, for speeding up the process, like introducing genes by transgenesis, even combining several R-genes simultaneously as reported by Zhu et al (2012), seems impossible at the moment in the country. The present constitution, approved in 2008 in Article No. 401 expresses; 'Ecuador is declared as free of seeds and genetically modified crops', so this technique cannot be used. The chance of using cisgenesis, which is defined as 'a crop plant that has been genetically modified with one or more genes (containing introns and flanking regions such as native promoter and terminator regions in a sense orientation) isolated from a crossable donor plant' (Schouten *et al.*, 2006), opens the possibility to help speeding up the breeding processes. But this technology needs to be approved legally in order to be applied in the country.

Another alternative, is represented by the technique of Gene Editing, which consists of site directed mutagenesis, using engineered site specific nucleases (SSNs) to delete, insert or replace a DNA sequence, leading to a change in the expression of the original gene without inserting alien DNA (Wiel et al, 2017). Originally, it was expected not to be subjected to restrictions like those for GMO's. Despite the apparent advantages it has, recently, this promising breeding technique is facing the same difficulties as with the GMO's since the European Union has judged that plants obtained with new mutagenesis techniques must be regulated by the same norms like for those derived from GMO procedures (Judgment. ECLI:EU:C:2018:583).

The most promising technique to help bring the potato breeding process in the country -not just for late blight but, for many other traits- to a higher level is the use of marker assisted selection (MAS). It will help to establish the presence and inheritance of known genes in a breeding population (Sliwka *et al.*, 2010) or in a germplasm collection (Alvarez et al, 2017).

Final considerations and future perspectives

The potato breeding by INIAP is a continuous challenge. Several superior characters need to be present in the new varieties. Together with late blight resistance and high yield, quality traits are demanded and also resistances to abiotic stresses have to be taken into account. Having said this, the major challenge remains resistance to the late blight disease. New sources for resistance to late blight have to be identified within the available germplasm collection or introduced into the country from abroad and must be efficient against the predominant genotypes of the pathogen.

Acknowledgements

To the Netherlands Organization for International Cooperation in Higher Education (NUFFIC), the Secretaria Nacional de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT), and the Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP) for the financial support. To Dr. Xavier Cuesta from INIAP's Potato Program for his valuable comments.

Literature cited

Albornoz, G., Ortuño, C. 1968. Santa Catalina: una variedad de papa para el centro de la Sierra ecuatoriana. Quito, Ecuador: INIAP. Boletín Divulgativo.

Alcoser, E. 2006. Caracterización de la demanda de papa en restaurants y asaderos de pollo ubicados en la zona centro sur de la ciudad de Quito. Tesis de Ingeniero Agrónomo. Tumbaco, Ecuador: Universidad Central del Ecuador.

Álvarez, M. F., Angarita, M., Delgado, M. C., García, C., Jiménez-Gomez, J., Gebhardt, C., Mosquera, T. 2017. Identification of Novel Associations of Candidate Genes with Resistance to Late Blight in *Solanum tuberosum* Group Phureja. Frontiers in Plant Science, 8, 1040. http://doi.org/10.3389/fpls.2017.01040

Andrade, H., Sola, M., Moreno, J., Borja, N., Alarcón, E. 1994. Mantenimiento, utilización y documentación de la colección ecuatoriana de papa. Revista INIAP

Andrade, H., Cuesta, X., Carrera, E., Yumisaca, F., Escobar, W., Yáñez, E. 1997. El papel del usuario en la selección y liberación de variedades en Ecuador. CIP Circular 22(3): 21-22.

Andrade, H. 2002. Perspectivas del mejoramiento de la papa en Ecuador: Algunas consideraciones para optimizar su producción y productividad. In Memoria XX Congreso de la Asociación Latinoamericana de la Papa (ALAP). 3-7 Junio de 2002. Quito, Ecuador Pp. 84-90.

Andrade, H., Bastidas, O., Sherwood, S. 2002. La papa en Ecuador. In Pumisacho M. & Sherwood S. (Eds.). El cultivo de la papa en el Ecuador. Quito, Ecuador: INIAP – CIP. Pp. 21-32.

Andrade, H., Sola, M., Moreno, J., Borja, N., Alarcón, E. 1994. Mantenimiento, utilización y documentación de la Colección Ecuatoriana de la Papa. Revista INIAP 4: 16-17.

Bastidas, G. 1991. Agricultores genetistas. In Memorias de la XV Reunión de la Asociación Latinoamericana de la Papa. P. 78.

Cañizares G., Forbes, G. 1995. Foliage resistance to *Phytophthora infestans* (Mont.) de Bary in the Ecuadorian national collection of *Solanum phureja* ssp. *phureja* Juz. & Buk. Potato Research 38: 3-10.

Capelo, J. 1998. Evaluación y selección de clones de papa con resistencia al tizón tardío (*Phytophthora infestans*) en campo de agricultores. Revista INIAP 6: 35-38.

Cárdenas, F.M., Andrade, H., Cuesta, X., Carrera, E. 1998. Evaluaciones participativas de calidad culinaria y aceptabilidad de clones promisorios de papa Solanum tuberosum L. en las Provincias de Chimborazo, Cotopaxi y Carchi del Ecuador. Revista INIAP

Chacón, G., Forbes, F. 2000. Evaluation of the resistance to *Phytophthora infestans* of two potato cultivars irradiated with gamma rays. Fitopatologia 35(4): 237-241.

Crissman, C.C., Cole, D.C., Carpio, F. 1994. Pesticide use and farm worker health in Ecuadorian potato production. American Journal of Agricultural Economics 76 (3): 593-597.

Cuesta, H., Andrade, J., Revelo, J., Andrade-Piedra, J., Garcés, S., Carrera, E. 1999. Obtención de clones de papa con resistencia horizontal a tizón tardío. Cartas de Sefit 7 (12): 11-12.

Cuesta, X., Castillo, C., Rivadeneira, J., Guevara, G., Vera, G. 2005. El mejoramiento genético de la papa en Ecuador. Revista Raíces Productivas 52: 10-11.

Cuesta, X, Andrade H, Carrera E, Montesdeoca, F. 2000. INIAP-Raymipapa e INIAP-Suprema: dos nuevas variedades de papa resistente a la lancha, precoces y de lata calidad culinaria. Revista INIAP.

Cuesta, X., Rivadeneira, J., Castillo, C., Reinoso, I. 2007a. INIAP-Estela. Quito, Ecuador: INIAP. Plegable No. 279.

Cuesta, X., Rivadeneira, J., Reinoso, I., Monar, C. 2007b. INIAP-Natividad. Quito, Ecuador: INIAP. Plegable No. 280.

Cuesta, X. Rivadeneira, J., Monteros, C., Yumisaca, F., Carrera, E., Reinoso, I. 2015. INIAP-Josefina: nueva variedad de papa con tolerancia a la sequía. Quito, Ecuador: INIAP. Plegable 420.

Cuesta, X., Rivadeneira, J., Monteros, C. 2015. Mejoramiento genético de papa: Conceptos, procedimentos, metodologías y protocolos. Quito, Ecuaodr: Instituto Nacional de Investigaciones Agropecuarias. 62 p.

Cuesta, X., Rivadeneira, J., Yumisaca, F., Carrera, E., Monteros, C., Reinoso, I. 2017. INIAP-Josefina: Nueva variedad de papa con tolerancia a la sequía. Revista Latinoamericana de la Papa 20 (2): 39 – 54.

Cuesta, X. Oyarzún, P.,Andrade-Piedra, J., Kromann, P., Taipe, A., Montesdeoca, F., Montesdeoca, L., Rivadeneira, J., Monteros, C., Monteros, C., Comina, P., Carrera, E., Reinoso, I. 2015. INIAP-Libertad: nueva variedad de papa con resistencia a lancha, precosidad y calidad. Quito, Ecuador: INIAP. Plegable 421.

Cuesta, X. 2011. Recursos genéticos de la papa y fitomejoramiento. In I Expo Congreso Nacional de Papa (Expopapa). 25-27 de noviembre de 2010 Ibarra, Ecuador.

Cuesta-Subia, X., Monteros-Altamirano, A. van den Berg, R., Visser, R.G.F., Vosman, B. 2012. Tuber quality characteristics of Ecuadorian potato landraces and farmer preferences. *Revista Latinoamericana de la Papa* 17(1): 125-151.

Delgado, R.A. & Vosman, B. 2009. Ecuadorian native potatoes a source for late blight resistance. Tropical Plant Pathology 34 (Suppl.): 245.

Delgado, R. A., Monteros-Altamirano, A. R., Li, Y., Visser, R. G. F., van der Lee, T. A. J., Vosman, B. 2013. Large subclonal variation in *Phytophthora infestans* populations associated with Ecuadorian potato landraces. Plant Pathology, 62: 1081–1088. doi: 10.1111/ppa.12039

Devaux A, Ordinola M, Hibon A., Flores R. 2010. *El sector papa en la región andina: Diagnóstico y elementos para una visión estratégica (Bolivia, Ecuador y Perú).* Lima, Perú: Centro Internacional de la Papa – CIP.

Espinoza, M., Andrade, H. 2007. Selección participativa de clones promisorios de papa (*Solanum* sp.) con resistencia al tizón tardío (*Phytophthora infestans*) proveniente de varias fuentes en dos localidades. 2006. Rumipamba 21(1): 1-14.

Estrada R., N. 2000. La biodiversidad en el mejoramiento genética de la papa. La Paz, Bolivia, PROINPA/CID/CIP.

Flores R, Viteri S., Sánchez MP, Galárraga J, Carpio J. 2013. Situación de Familias Productoras de Papa en la Sierra Central del Ecuador: Línea de Base Productiva del Proyecto IssAndes. Documento de trabajo – Ecuador No. 2. Proyecto "Fortalecimiento de la innovación agrícola pro pobre para la seguridad alimentaria en la región andina – IssAndes". OFIAGRO, Centro Internacional de la Papa. Quito. 107 p

Forbes GA, Escobar XC, Ayala CC, Revelo J, Ordoñez ME, Fry BA, Doucett K, Fry WE, 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. *Phytopathology* 87, 375-380.

Garófalo J, Andrade H, Cuesta X, 2006. Evaluación de la aptitud combinatoria general y específica en 21 progenies de papa Solanum phureja para resistencia a tizón tardío Phytophthora infestans. In II Congreso ecuatoriano de la papa. May 17 – 19, 2006. Ambato, Ecuador.

Lagerheim, G. 1890. La enfermedad de los pepinos, su causa y su curación. Revista Ecuatoriana 2: 484-488.

López, R., Yánez, E. 2010. Evaluación y selección de plantas mutantes con resistencia a tizón tardío (*Phytophthora infestans*) en variedad Superchola (*Solanum* spp.) a nivel de campo obtenidas mediante radiaciones ionizantes gamma en Mejía, Provincia de Pichincha. 4to Congreso Ecuatoriano de la Papa.

Mayorga, S. 2006. Caracterización de la demanda de papa en restaurants y asaderos de pollo ubicados en la zona centro norte de la ciudad de Quito. Tesis de Ingeniero Agrónomo. Tumbaco, Ecuador: Universidad Central del Ecuador.

Monteros-Altamirano, A. 2011. Potato landraces: description and dynamics in three areas of Ecuador. PhD. Thesis. Wageningen, The Netherlands, Wageningen University.

Monteros-Guerrero, A. 2016. Rendimientos de papa en el Ecuador. Primer ciclo 2016. Quito: Ministerio de Agricultura, Ganadería y Pesca. 10 p. http://sipa.agricultura.gob.ec/descargas/estudios/rendimientos/papa/rendimiento_papa_2016.pdf

Morales, R.; Revelo, J.; Suárez, G.; Forbes, G. Muñoz, F. 1995. Relación entre el ataque de *Phytophthora infestans* y la producción en el cultivo de papa (*Solanum tuberosum*). In Memorias XVII Reunión de la Asociación Latinoamericana de la Papa (ALAP). Mérida, Venezuela. Pp. 73-74.

Navarrete I, Panchi N, Andrade-Piedra JL. 2017. Potato crop health quality and yield losses in Ecuador. Revista Latinoamericana de la Papa 21 (2): 69 – 88.

Oyarzún P., Gallegos P., Asaquibay, C. Forbes G., Ochoa J., Paucar B., Prado M., Revelo J., Sherwood S., Yumisaca F. 2002. Manejo integrado de plagas y enfermedades. In Pumisacho M. & Sherwood S. (Eds.). El cultivo de la papa en el Ecuador. INIAP - CIP:
85-131.

Pachano, A. 1918. Dos enfermedades de las papas. Quinta Normal Estación Experimental Circular 7: 1-11.

Pumisacho, M. & Velázquez, J. 2009. Manual del cultivo de papa para pequeños productores. Quito, Ecuador: INIAP-COSUDE.

Reinoso, I. 1994a. Variedades de papa comercializadas en Quito, Guayaquil y Cuenca, durante 1992. Revista INIAP 1: 34-35.

Reinoso, I. 1994b. Comercialización de papa en tres mercados urbanos del Ecuador. Revista INIAP 5: 49-50.

Revelo, J., Garcés, S., Andrade, J. 1997. Resistance of commercial potato varieties to the attack by *Phytophthora infestans* in Ecuador. CIP Circular: 20-21.
Revelo J., Garcés S., Andrade J. 1997. Identification of sources of horizontal resistance to *Phytophthora infestans* free of major genes. CIP Circular (April): 15-17.

Revelo, J., Andrade-Piedra, J., Garcés, S. 1997. El tizón tardío o lancha de la papa: problema permanente de los agricultores. In Memorias del Curso: 'Manejo integrado de las principales plagas y enfermedades del cultivo de la papa'. Pp. 14-16. Quito, Ecuador, INIAP.

Schilde, L. 2003. Focus on partners: The INCO-PAPA Project. GILB Newsletter 20: 1-2.

Sherwood, S. G. 2009. Learning from Carchi: Agricultural Modernization and the Production of Decline. PhD. Dissertation. Wageningen, Netherlands: Wageningen University.

Sliwka, J., Jakuczun, H., Kamiński, P., Zimnoch-Guzowska, E. 2010. Marker-assisted selection of diploid and tetraploid potatoes carrying *Rpi-phu1*, a major gene for resistance to *Phytophthora infestans.* Journal of Applied Genetics 51(2), 133–140.

Sola, M. 1986. El fitomejoramiento en la obtención de nuevas variedades de papa. In Quinto Curso sobre producción de semilla de papa a partir de cultivo de tejidos, mejoramiento y tecnología del cultivo. 12 -14 Marzo, 1986. Quito, Ecuador; INIAP-UC-CIP-PRACIPAPA.

Sola, M. 1987. El programa de papa ecuatoriano. In Ramakrishna, B. (ed.) II Seminario Nuevos enfoques para mejoramiento de la papa. Quito, Ecuador, IICA-PROCIANDINO. Pp. 117-126.

Taipe, A., Oyarzun, P., Cuesta, X., Rivadeneira, J. 2002. Generación de variedades con resistencia duradera al tizón tardío (*Phytophthora infestans*). Revista INIAP 5: 22-24.

Vincelli, P. 2016. Genetic Engineering and Sustainable Crop Disease Management: Opportunities for Case-by-Case Decision-Making. Sustainability 8, 495. https://doi.org/10.3390/su8050495

Wiel, C.C.M., Schaart, J.G., Lotz, L.A.P., Smulders, J.M.J. 2017. New traits in crops produced by genome editing techniques based on deletions. Plant Biotechnology Reports11(1): 1–8. doi: 10.1007/s11816-017-0425-z

Yánez, E., Espinoza, P., Sevillano, C., Yumisaca, F., Guerrero, D., Cuesta. X. 2008. Estudio de línea base de variedades de papa en comunidades de la provincia de Carchi, Chimborazo y parroquia Quero en Ecuador. In XXIII Congreso de la Asociación Latinoamericana de la Papa (ALAP). Pp.441-442.

Zhu, S., Li, Y., Vosse, J.H., Visser, R.G.F., Jacobsen, E. 2012. Functional stacking of three resistance genes against Phytophthora infestans in potato. Transgenic Research 21(1): 89–99. doi: 10.1007/s11248-011-9510-1

Figure 1. View of a potato field in Pichincha province at 3000 masl.

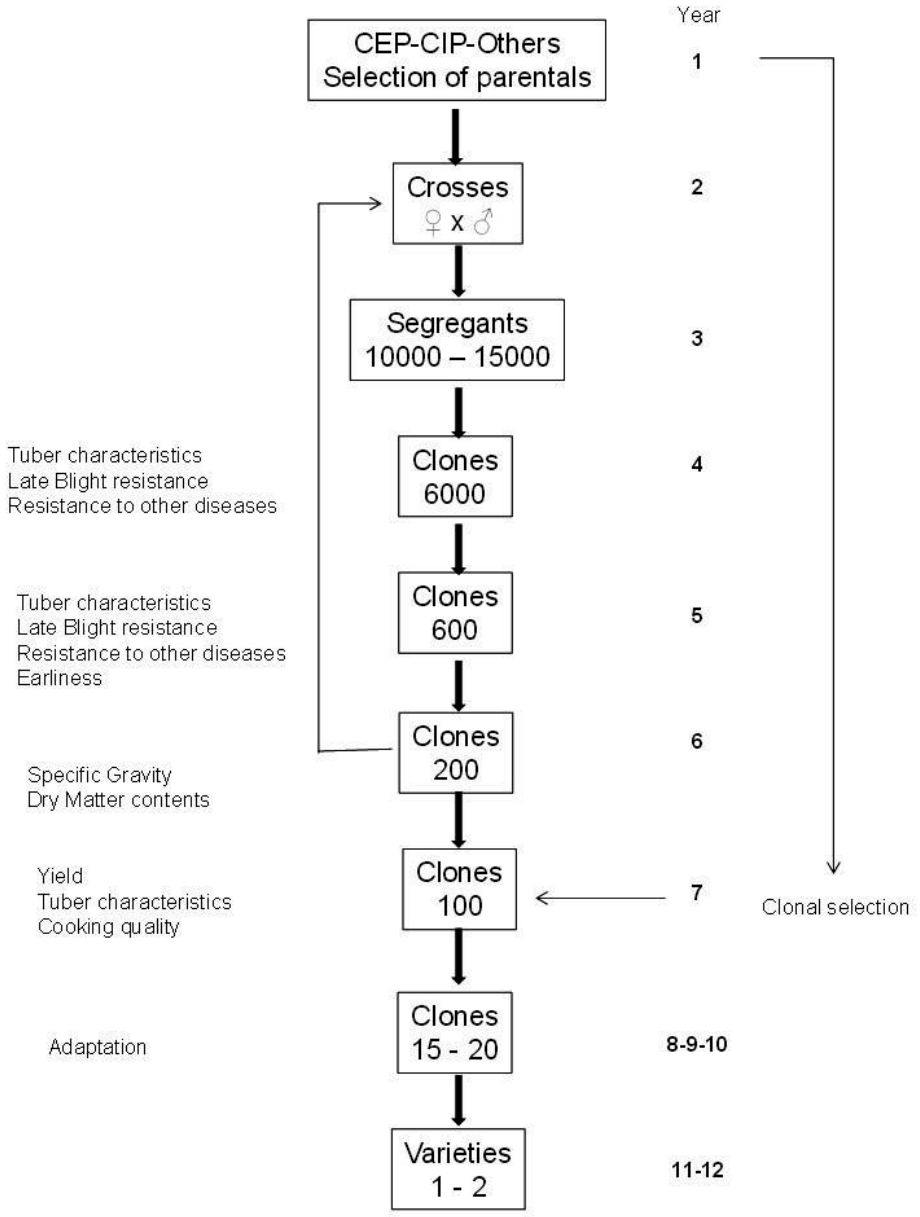


Figure 2. Breeding processes carried out by the Potato Program of INIAP (Adapted from Cuesta, 2011 & Cuesta et al, 2015).

Figure 3. Morphological variability of potato landraces (shape, color, eye depth).

Figure 4. Field plot for selection of clones.

Table 1. Potato varieties released by INIAP in Ecuador.

Name	Genealogy	Release	Potential Yield (t/ha)	Resistance to Late blight	Presence of R genes*
INIAP-Santa Catalina	(Branca Cascuda x Pana Blanca) x (Jabonilla x Curipamba)	1965	30	R	N
INIAP-María	Black x (Paspuela x Leona)	1967	35	MR	Y
INIAP-Cecilia	Vertifolia (*S. tuberosum*) x Jabonilla (*S. andigena*)	1967	25	HS	Y
INIAP-Gabriela	Algodona x Chola	1982	36	S	Y
INIAP-Esperanza	Florita x Chola	1983	38	S	Y
INIAP-Fripapa	(Bulk México x 378158.721) x I-1039	1995	40	R	Y
INIAP-Rosita	(Nevada x I-1058) x Bulk Mexico	1995	50	S	Y
INIAP-Santa Isabel	Chola x (Jabonilla x Curipamba)	1995	40	S	Y
INIAP-Margarita	(Bulk LLT-Pop x 378493.928) x IVPCE 10	1995	40	R	Y
INIAP-Soledad Cañari	Atzimba x Chola	1996	30	S	Y
INIAP-Raymipapa	378979.46 (CCCU-69.1 x Bulk Seedl.78 Mx) x Bulk Seedl 79/80 Mex	1999	40	R	Y.
INIAP-Suprema	(ABPT) B.2 x bk (LB78.79).	1999	30	HR	Y
INIAP-Papa pan	Unknown	2000	40	HR	Y
INIAP-Estela	Superchola x (Yema de Huevo (*S. phureja*) x *S. pausissectum*)	2007	40	R	n.d.
INIAP-Natividad	INIAP-Gabriela x (Yema de Huevo (*S. phureja*) x *S. pausissectum*)	2007	45	MR	n.d.
INIAP-Santa Ana	Superchola x INIAP-Fripapa	2007	30	MR	n.d.
INIAP-Victoria	INIAP-Gabriela x INIAP-Fripapa	2011	40	MR	n.d.
INIAP-Puca Shungo	BOM-532 x BOM-532	2011	27	MR	n.d.
INIAP-Yana Shungo	HSO-213 x HSO-213	2011	25	MR	n.d.
INIAP-Josefina	Bolona x (*S. phureja*) x *S. pausissectum)*	2015	18-36	MR	n.d.
INIAP-Libertad	B3C0	2015	25-48	R	n.d

R = Resistant; MR= Moderately resistantant; HR= Highly resistant; S= Susceptible; HS= Highly susceptible. * N =no; Y= yes.

Table 2. Tuber characteristics of important Ecuadorian potato varieties.

Name	Release	Skin	Flesh
INIAP-Santa Catalina	1965	Cream	Yellow
INIAP-María	1967	Cream	White
INIAP-Cecilia	1967	Cream	White
INIAP-Gabriela	1982	Pink	Cream
INIAP-Esperanza	1983	Cream	Cream
Superchola	1984	Pink	Yellow
INIAP-Fripapa	1995	Pink	Yellow
INIAP-Rosita	1995	Red	Yellow
INIAP-Santa Isabel	1995	Red	Yellow
INIAP-Margarita	1995	Yellow	Cream
INIAP-Soledad Cañari	1996	Cream	Yellow
INIAP-Raymipapa	1999	Cream	Yellow
INIAP-Suprema	1999	Cream	White
INIAP-Papa pan	2000	Cream	White
INIAP-Estela	2007	Purple	Yellow
INIAP-Natividad	2007	Yellow	Yellow
INIAP-Santa Ana	2007	Yellow	Yellow
INIAP-Victoria	2011	Red	Yellow
INIAP-Puca Shungo	2011	Purple	Red
INIAP-Yana Shungo	2011	Brown	Purple
INIAP-Josefina	2015	Red	Yellow
INIAP-Libertad	2015	Yellow	Cream

Chapter 3

Large sub-clonal variation in *Phytophthora infestans* populations associated to Ecuadorian potato landraces

Ricardo A. Delgado[1,2,4], Alvaro Monteros-Altamirano[1,2], Ying Li[3], Richard G.F. Visser[2], Theo A.J. van der Lee[3], Ben Vosman[2]

[1] Instituto Nacional Autónomo de Investigaciones Agropecuárias (INIAP), Estación Experimental Santa Catalina, Panamericana Sur km 1, Quito, Ecuador.
[2] Plant Breeding, Wageningen University and Research, P.O. Box 386, 6700 AJ, Wageningen, The Netherlands.
[3] Plant Research International, Biointeractions and Plant Health, Wageningen University & Research, P.O. Box 16, 6700 AA Wageningen, The Netherlands
[4] Graduate school Experimental Plant Sciences, Wageningen University

Published in Plant Pathology (2013) 62, 1081–1088

Abstract

The population of *Phytophthora infestans* on potato landraces in three provinces (Carchi, Chimborazo and Loja) of Ecuador was analysed. All isolates (n= 66) were of the A1 mating type. SSRs were used to assess the genetic diversity of the isolates. The *P. infestans* isolates from the potato landraces grouped in a single clade together with reference isolates belonging to the clonal lineage EC-1. In the 66 SSRs profiles obtained, 31 multilocus genotypes were identified. The 66 isolates, constituted 49 different races according to the *S. demissum* differential set (*R1* to *R11*). The *P. infestans* population observed was complex and virulent on 4 to 11 *R*-genes. Analysis showed that the sub-clonal variation in the Ecuadorian EC-1 clone is increasing over time and is much larger than clonal variation in lineages in the Netherlands and Nicaragua, suggesting high mutation rates and little or no selection in Ecuador.

Keywords: Late blight, SSRs, diversity, virulence, *P. andina*

Introduction

The oomycete *Phytophthora infestans* is the causal agent of late blight and attacks a range of species in the *Solanaceae* family. It is the most important disease on potato in Ecuador and worldwide (Oyarzún *et al*., 2002). This species had been studied worldwide using phenotypic and genotypic markers. Among the phenotypic markers, the mating type and virulence had been used. The mating type is based on the fact that P. infestans is heterothallic so two types are necessary for sexual reproduction, named A1 and A2. For virulence studies, the R gene differential set is used. This is based on the reaction of *P. infestans* isolates with *Solanum* plants carrying specific resistance genes, and grouping those with the same virulence phenotype as races. Among the genotypic markers, allozymes, restriction fragment length polymorphisms (RFLPs), mitochondrial haplotypes, amplified fragment polymorphisms (AFLPs) and simple sequence repeats (SSRs or microsatellites) had been used (Fry *et al*., 2009). Studies on *Phytophthora* associated with *Solanaceae* in Ecuador, showed the presence of four clonal lineages based on RFLPs fingerprinting pattern (Forbes *et al.*, 1997, Adler *et al*., 2004). Two clonal lineages, EC-1 and US-1, both of the A1 mating type have been identified already in the previous century (Forbes *et al*., 1997). The EC-1 lineage is predominant on potatoes and the US-1 on tomatoes (Forbes *et al*., 1997; Oyarzún *et al*., 1998). More recently, two additional lineages were found in Ecuador, EC-2 and EC-3, which cause late blight on non-tuber bearing *Solanum* species, such as *S. betaceaum*, *S. quitoense*, *S. hispidum*, and *S. muricatum* (Adler *et al*.., 2004; Ordoñez *et al.*, 2000). Oliva *et al.,* (2010) formally described EC-2 and EC-3 as a distinct species *Phytophtora andina.* However, the species status of *P. andina* is questioned by others (Cárdenas *et al.,* 2012), it may actually be a hybrid between *P. infestans* and a yet unknown lineage. Support for this also comes from a study by Blair *et al.,* (2012).

Studies on the race structure of *P. infestans* populations isolated from cultivated potatoes in Ecuador showed an increase in the complexity from a predominance of avirulent isolates (INIAP, 1974, INIAP, 1975, INIAP, 1976) to complex races, which was suggested to have resulted from the replacement of the US-1 on potatoes by the EC-1 lineage (Forbes *et al.*, 1997). Additionally, EC-1 was found to be more aggressive on potato than isolates of the US-1 clonal lineage (Oyarzún *et al*., 1998). Forbes *et al.,* (1997) found twenty-four races infecting potatoes in Ecuador, 14 in Carchi, 14 in Chimborazo and 8 in Loja. More recently, in commercial varieties and selected clones from the INIAP's national potato breeding program, 27, 17 and 37 races were found in Carchi, Cotopaxi and Pichincha provinces, respectively (Tello, 2008). In Perú and Colombia three clonal lineages were identified that infected potato landraces (Garry *et al*., 2005, Vargas *et al*., 2009) and, as in Ecuador the dominant lineage was EC-1. Despite all previous studies on *Phytophtora* species, there are no specific studies on populations of *P. infestans* associated with Ecuadorian potato landraces and it is unknown if there are any other lineages or species present on them.

There are more than 400 potato landraces in Ecuador (Cuesta *et al*., 2005). These landraces are the result of selection and conservation carried out by small scale farmers in the highlands (Cuesta *et al*., 2005). In contrast to conventional potato cultivation these potatoes are cultivated on small acreage, with low input of pesticides and often several landraces together (Cuesta *et al.*, 2005, Monteros & Reinoso, 2010). Three species had been observed among the landraces cultivated nowadays: the diploid *Solanum phureja*, the triploid *S. chaucha* and the

tetraploid *S. andigena* (Monteros-Altamitano, 2011). They constitute a potential source of genetic variation for breeding purposes, in characters such as quality, earliness and resistance to biotic and abiotic stresses. Evaluations showed that most landraces in Ecuador were highly susceptible to late blight, but some landraces with field resistance to late blight were also identified (Cañizares & Forbes, 1995; Revelo *et al.*, 1997, Monteros-Altamirano, 2011).

The aims of this study were to characterize *P. infestans* populations associated with Ecuadorian potato landraces in three areas of Ecuador, to compare these to populations previously reported on commercial potatoes; and to assess the impact of landraces on the *P. infestans* population. We also compared the genotypic diversity found in the EC-1 lineage present in Ecuador to the variation found elsewhere.

Materials and methods

Isolate collection

Phytophthora infestans isolates were collected from potato landraces present in three regions of Ecuador: in the provinces of Carchi in the north, Chimborazo in the centre and Loja in the south (Monteros *et al*., 2008). Of each potato landrace present on a farm, five to ten leaves with a single lesion were sampled. Leaves were kept at 4 °C until isolation (Forbes *et al*., 1997). The name and ploidy level of the landrace sampled, farm owner, location, GPS-coordinates and altitude were recorded (Table S1). The pathogen was isolated either from infected leaves or from small pieces of necrotic leaves, which were placed between potato slices of the susceptible variety Superchola, in both cases incubated in a humid chamber at 16 ^{o}C, with a 12 hours photoperiod. Once mycelium was visible on leaves or slices, it was transferred to Petri dishes with Rye B Agar medium (Caten & Jinks, 1968) with antibiotics (Oyarzún *et al*., 1998). Purified isolates of *P. infestans* were maintained on Rye A medium (Caten & Jinks, 1968).

Isolate characterization

The mating type was determined for each isolate by pairing it with known A1 (EC3090 or EC3690) and A2 (EC3260) isolates (provided by International Potato Center (CIP), Lima, Peru) on 10% clarified V8 agar (Forbes, 1997) at 18 °C. After 2 to 3 weeks each plate containing the paired isolates was assessed for the presence of oospores. Isolates that produced oospores in the presence of a known A2 tester were designated as the A1 mating type, and vice versa (Forbes, 1997).

Virulence was determined using a differential set of potato clones containing 11 major *P. infestans* resistance genes from *S. demissum* (Malcomson & Black, 1966). R1 (CIP 801038), R3 (CIP 801041), R4 (CIP 801042), R5 (CIP 801043), R7 (CIP 801045), R8 (CIP 801046) and R9 (CIP 800994) differential cultivars were provided by CIP. R2 (CIP 800987), R6 (CIP 800991), R10 (CIP 800995) and R11 (CIP 800996) differentials, and the susceptible cv. Bintje which has no known R genes (Montarry *et al*., 2010) were obtained from Wageningen University and used as control. Each isolate was inoculated on the differentials. From each

plant, three leaflets were taken and placed in inverted Petri dishes containing Water-Agar. On the abaxial surface of each leaflet, two 20 µL drops containing 25 x 10^3 sporangia/mL were placed at each side of the mid vein. The inoculated leaves were placed in a climate chamber at 16 °C for six days with 12 hours of photoperiod, after which the reaction was scored. The reaction was considered compatible, when a necrotic lesion and/or sporulation was observed on a leaflet, and as incompatible when hypersensitive reaction was seen or no lesion was visible.

DNA extraction

All the *P. infestans* isolates obtained from potato landraces were grown from 10 – 14 days on Pea broth (Forbes, 1997). The mycelium was harvested and lyophilized. DNA was extracted from each sample with AGOWA sbeadex ® Maxi Plant kit on a KingFisher96 robot (Thermo Fisher Scientific).

SSR amplification and genetic data analysis

Twelve SSRs were used in this study, including Pi04, Pi63, Pi70, G11, D13, Pi4B (Lees *et al*., 2006), PinfSSR2, PinfSSR3, PinfSSR4, PinfSSR6, PinfSSR 8 and PinfSSR11 (Li *et al*., 2010). They were selected from previously published sets according to their map position, ease of scoring and allelic diversity (Knapova & Gisi, 2002, van der Lee *et al.,* 2004, Lees *et al.*, 2006, Li *et al.,* 2010). Amplifications were run in a PTC200 thermocycler (MJ Research, Waltham, Massachusetts, USA), with an initial denaturation at 95°C for 15 min, followed by 30 cycles of 95°C for 20 sec, 58°C for 90 sec, and 72°C for 60 sec, and a final extension at 72°C for 20 min (Li, 2012). In the analysis we included a number of isolates from previous surveys carried out in Ecuador for reference (Table S2). The resulting amplification products were sized by capillary electrophoresis on an ABI 3730 sequencer using the molecular standard GeneScan-500 ROX and scored using GeneMapper 3.7 software (Applied Biosystems, USA). Within the GeneMapper software kits, panels and binsets were generated defining the markers and their known allele bins, which were provided by Plant Research International (PRI, Wageningen, the Netherlands). The known panels and binsets for the 12 SSRs have been used to size SSR alleles in thousands of isolates from different countries by PRI and the James Hutton Institute (JHI, UK). Most *P. infestans* isolates showed a maximum of two alleles per locus, as expected for a diploid organism. However, in a number of cases more than two alleles were found, which may result from aneuploidy or polyploidy (see for examples Additional Figure 1). This complicates the analysis as the analysis tools assume haploid or diploid data. Therefore, we converted the detected SSR fragments (alleles) to binary presence (1) or absence (0) data. From this a Neighbour Joining tree was produced using PAUP* 4.0 Beta software. Robustness of the phylogram branches was inferred from Jacknife values after 10.000 replicates (Swofford, 2002).

Diversity analysis

Race and genotypic diversity were estimated using the Shannon index (H_s) as $H_s = -\sum (p_i . \ln p_i)$, where p_i is the frequency of the race or genotype. The Evenness (E) was estimated by the formula: $E = H_s / \ln(n)$, where n is the total number of isolates of the sample and H_s is the Shannon index. E varies 0 from to 1 with 1 representing a situation in which all races or genotypes are equally abundant (Magurran, 1988).

The significance of different Shannon indices was assessed with the t-test of Hutcheson (t_H) (Hutcheson, 1970). It was calculated as follows: $t_H = (H_1\text{-}H_2)/[S^2_{H1}+S^2_{H2}]^{1/2}$, where $H_1\text{-}H_2$ is the difference among the Shannon indexes between two samples. The variance of each sample (S^2) was estimated with the formula: $S^2 = [\sum p_i.(\ln p_i)^2 - (\sum p_i.\ln p_i)^2/n]/n^2$, p_i and n are as described above.

The degrees of freedom (*df*) for each pairwise comparison were calculated using the formula: $df=[S^2_{H1}+S^2_{H2}]^2/[((S^2_{H1})^2/n_1) + ((S^2_{H2})^2/n_2)]$.

To examine the distribution of genetic variation within and among populations, analysis of molecular variance (AMOVA) using Arlequin version 3.5 (Excoffier *et al.*, 2005) was carried out with the binary dataset as input. Population differentiation (Fst) was calculated using Arlequin.

To compare the sub-clonal variation in the Ecuadorian to other populations, the genotyping data of 219 Dutch isolates was used. These isolates were collected during the period 2004-2009 in the Netherlands and genotyped with the same 12 SSR markers. The genotyping data and isolate information can be found in Li *et al.* (2012a). In addition we calculated the Shannon index and the Evenness using the frequency data in Nicaragua reported by Blandón-Díaz (2011). These were based on four multilocus genotypes obtained with six SSRs on 165 *P. infestans* isolates collected from potatoes in Nicaragua.

Results

Isolate collection

A total of 66 *P. infestans* isolates were collected during the years 2009-2010 from 16 farms (Table 1), details on these isolates can be found in Table S1. The reason for the low number collected in Loja was the severe drought that occurred in that province at the time of collection, making it difficult to obtain samples.

Isolate characterization using potato R gene differentials

All isolates were of the A1 type mating type. The number of races collected and associated virulence factors are also shown in Table S3. There were 49 different races in total (Table 1). Twenty seven races were identified in Carchi, 18 in Chimborazo and seven in Loja. The *P. infestans* population observed was complex, and virulent on 4 to 11 R-genes. One race (1,3,4,5,6,7,8,9,10,11) was present in all three provinces. The rest was restricted to one site (Table S3).

Isolate characterization using SSR markers

Eight of the twelve SSR markers were polymorphic among the *P. infestans* isolates collected. Markers Pi04, Pi63, Pi70 and PinfSSR8 were monomorphic (Table S4).

For some markers we observed three or even four alleles in a particular isolate. In the *P. infestans* isolates from potato landraces, 3 alleles were observed with Pi4B and PinfSSR4 markers (Table S2). In the reference *P. infestans* isolates, 3 alleles were detected with markers Pi4B, Pi63, PinfSSR4 and PinfSSR8 (Table S5). In the case of *P. andina*, three to four alleles were detected with Pi4B marker (Table S5). Several private and monomorphic alleles were detected. Allele 279 from locus Pi63 was detected in all *P. infestans* isolates. Allele 188 from locus Pi70 was present in all US-1 reference isolates and absent in the rest of *P. infestans* isolates. In total we observed 31 different genotypes among the 66 isolates (Table 1) using the SSRs.

Relationship among the materials collected

The phylogenetic tree obtained from the SSR profiles, showed that the *P. infestans* isolates grouped together in a large clade (Figure 1; the structure analysis is shown in Figure S2). This clade also included the reference isolates known to belong to the clonal lineage EC-1. They were clearly distinct from the reference isolates identified as US-1 and *P. andina*. We did not find a relation among the multilocus genotypes and race phenotype. Isolates that belonged to the same race could show different multilocus genotypes and the other way around. Even isolates obtained from the same landrace and sharing the same virulence phenotype, could belong to different SSR multilocus genotypes (Table S1).

Diversity among regions and development of diversity over time

In total, 31 different multilocus genotypes were identified. From all, just one (EC-1_001) occurred in the three provinces, but predominated in Carchi. Another one (EC-1_003) was found in two provinces (Carchi and Chimborazo). The rest was restricted to one province (Table S4). To examine the distribution of genetic variation, among and within sub-populations as defined by the three geographical regions (Carchi, Chimborazo and Loja), AMOVA was performed. This showed that, 82% of the variance was present within sub-populations whereas the remaining 18% among the 3 populations. There was a clear relation between genotype and geographic origin, as the Fst value was 0.18 ($P < 0.0001$).

Carchi had the highest Shannon diversity index for race phenotype (3.15) and it was significantly more diverse than Chimborazo (t_H=23.28, df=46.29, p<0.0001) and Loja (t_H= 7.31, df=15.32, p<0.001) (Table 1). Chimborazo, with a Shannon Index of 2.86, was more diverse than Loja (t_H=17, df= 17.13, p<0.0001), which showed the lowest diversity value (1.89). The Evenness among provinces showed slight, non-significant differences.

Based on the SSR markers, the *P. infestans* population in Carchi (H_s =2.44) was genetically more diverse than the population in Chimborazo (t_H=18.56, df= 30.39, p<0.001) and Loja (t_H=5.48, df= 9.43, p<0.001). The populations in Chimborazo and Loja were not significantly different from each other (t_H=-0.80 df= 10.73, p=0.372) (Table 1). The total race diversity ($H_{s=}$ 3.78, Table 1) was significantly higher than the genotypic diversity (H_s = 3.03, Table 1) (t_H=12.66, df= 66.28, p=0.007). When analysing the genotype changes over the time in the Ecuadorian population of *P. infestans*, there is increase in diversity, evenness and number of genotypes (Table 2).

Comparison of the variation within the EC-1 in Ecuador to other clonal lineages.

We also compared the genetic variation within the Ecuadorian population (the 3 regions taken together) with the variation in a clonal lineage that is dominant in Europe. In total 219 Dutch isolates from the clonal lineage Blue_13 were studied with the same set of SSR markers (Li *et al.,* 2012..). The markers split Blue_13 clonal lineage in 32 different subclones. The genotyping diversity (*Hs*=1.98, *E*=0.37) in the Dutch clonal lineage is significantly (t_H = -5.00, df =177.88, p = 1.36E-06) lower than the diversity (*Hs*=3.03, *E*=0.72) in the Ecuadorian population. Also when we calculate the diversity present in the Nicaraguan population based on the data provided by Blandón-Diaz (2011), (165 isolates, 5 SSR multilocus genotypes *Hs*=0.23, *E*=0.04) we see that the variation in this population is also much lower than in the Ecuadorian population.

Discussion

Sixty-six isolates of P. infestans were obtained from 16 farms in three provinces of Ecuador. As a first step in the characterization, their virulence was analyzed on a set of differential genotypes containing the P. infestans resistance genes R1 to R11 (Black et al., 1953; Malcolmson & Black, 1966), as these genes and functional homologues are widely distributed in Solanum spp. (Vleeshouwers *et al.*, 2011). Using this differential set of genotypes, 49 (75%) of the isolates were found to be unique races. Simple races capable of overcoming one to three R genes were not observed. A similar high frequency of different races among isolates has been reported from Costa Rica (37 out of 40; Barquero *et al.*, 2005). However, lower frequencies were observed in other studies, e.g. Nepal 30 out of 251, Estonia 86/432, Finland 66/269, Norway 38/105, Nordic countries (Denmark, Finland, Norway and Sweden) 31/177 and China 61/125 races (Hermansen *et al.*, 2000; Ghimire *et al.*, 2001; Lehtinen *et al.*, 2008; Li *et al.*, 2009; Runno-Paurson *et al.*, 2010). It should be noted that in Denmark, Estonia, Finland, Norway and Sweden sexual populations are dominant, and the populations seems to be clonal in China and Nepal (although both mating types can be found), whereas the population in Ecuador is clonal.

The variation in the Ecuadorian population may be maintained due to a lack of selection for a particular race. Most of the Ecuadorian potato landraces are highly susceptible to late blight (Cañizares & Forbes, 1995; Revelo *et al.*, 1997; Monteros-Altamirano, 2011). In addition, fungicides are rarely used in the small-scale farming system used for these potato landraces. Grünwald *et al.,* (2006) reported that populations of *P. infestans* not exposed to the fungicide metalaxyl showed more genetic diversity than exposed ones.

The current study has confirmed that *P. infestans* attacking potato in the Ecuadorian highlands is of the EC-1 clonal lineage. Notwithstanding the fact that all isolates from potato landraces belonged to the same clonal lineage, genetic variation was detected using the SSRs and by phenotypic characterization. Thirty-one multilocus SSR genotypes were detected among the 66 isolates. This variability is large for a clonal lineage. Clonal lineages have been identified in Europe (Cooke *et al.*, 2006, 2012) and Nicaragua (Blandón-Díaz *et al.*, 2012) using microsatellites. Guo *et al.,* (2009) identified a single clonal lineage in northern China using

two SSRs; Li *et a.,*. (2012b) identified three clonal lineages in western China using ten SSRs. In the Nordic countries (Denmark, Finland, Norway and Sweden), 169 SSR multilocus genotypes were identified using seven microsatellite markers from a sample of 191 P. infestans isolates. This high genetic diversity observed in Nordic countries is attributed to the sexual reproduction of the pathogen (Brurberg *et al.*, 2011). Comparing the results of the current study to published data is not easy as different sampling strategies and different numbers of SSR markers have been used: the best comparison is with the European Blue_13 lineage as this study was carried out with the same set of SSR markers. The variation in the Ecuadorian EC-1 lineage was significantly greater than the diversity in Blue_13 as measured by the Shannon index and evenness. When the results of the current study are compared to the population present in Nicaragua (only considering markers that were present in both studies), the variation in the Ecuadorian population was greater than that in the Nicaraguan population. The high diversity in Ecuador might be related to a high mutation frequency, e.g. due to increased UV radiation as potatoes are grown in Ecuador at high altitudes (>2400 m). It is also possible that the greater diversity in the EC-1 population than in the clonal lineage Blue_13 is due to the age of the population: Blue_13 was detected for the first time in samples from 2004 in the Netherlands (Li *et al.*, 2012a), whereas EC-1 was described in the 1990s (Forbes *et al.*, 1997).

Mechanistically, the subclonal variation may partly be explained by loss of chromosome regions or mitotic recombination. Nevertheless, new alleles were detected that can only be explained by changes in the number of repeat units during mitosis. The presence of more than two peaks in some of the SSR profiles supports the hypothesis that polyploidization or gene duplication followed by mutations caused the genetic diversity observed in the *P. infestans* population of Ecuador (Tooley & Therrien, 1991; Cooke *et al.*, 2012). The occurrence of more than two alleles at a specific locus has been reported before (Knapova & Gisi, 2002; Lees *et al.*, 2006; Chacón Acosta, 2007; Akino *et al.*, 2009; Oliva Pérez, 2009; Cooke *et al.*, 2012; Li *et al.*, 2012a,b). The same mechanism seems to be active in *P. andina* also, where four alleles were observed (Fig. S1). Changes in virulence spectrum have been attributed to (partial) chromosomal deletions (van der Lee *et al.*, 2001). Also, mutations in avirulence genes have been found to cause changes in the virulence phenotype (Armstrong *et al.*, 2005). Copy number variations, amino acid replacements, and gene gains and losses have been suggested as sources of the variability within the clonal lineage Blue_13 in the UK (Cooke *et al.*, 2012). The high race and SSR diversity observed in the Ecuadorian population might be related to the cultivation of potatoes all year round, so several generations of the pathogen can occur, increasing the chance of the appearance of new genotypes.

There was no correlation between the phenotypic and genotypic diversity. Isolates of a particular race showed different multilocus genotypes. These occurred even in isolates obtained from the same landrace or farm (Table S1). Thus, the observed variation appears to be produced randomly with no selective pressure. The high race and SSR diversity observed in this study contrasts with populations from USA, France and China, where clonal lineages were shown to possess a wide diversity in races but a low genetic diversity (Abu-El Samen *et al.*, 2003; Montarry *et al.*, 2006; Guo *et al.*, 2009).

The genetic diversity was highest in the Carchi province and significantly different from Chimborazo and Loja (Table 1). The FST analysis showed clear differentiation of the populations in the three regions and most of the variation was present within populations. This difference in diversity observed in Carchi could be due to the higher number of isolates and farms sampled. The highest evenness value was measured in Loja (0.93), although the number of samples was small (Table 1) and the value probably reflects under-sampling. The one genotype that occurred in all three provinces might have migrated through the Ecuadorian highlands, perhaps as a result of the exchange of landrace seeds among farmers (Monteros-Altamirano, 2011). Alternatively, this genotype may have arisen multiple times independently. The microsatellite analysis also showed some P. infestans lineage- and species-specific alleles. These may be used to distinguish clonal lineages (Akino *et al.,* 2009). The SSR markers used in this study reflected the genetic diversity within the current P. infestans population in Ecuador: in future they may be used to monitor changes in the population and displacement of pathogen genotypes across the country.

Comparisons with previous studies have to be treated with caution because of the different sampling strategies used. However, an increase in the Shannon index and evenness over time was apparent, as was an increase in the number of races. The differences in Carchi in 2007 and 2009–2010 may be due to the fact that the present study survey included eight farms, whereas Tello (2008) collected all the samples from just one farm in the province and some races were much more frequent than others. The evenness values are close to 1 for all provinces, which was not the situation in 1990–1993, where in Carchi two races represented 40% of the isolates. For Chimborazo and Loja, the situation was similar, two races at each location representing 55 and 70% of the isolates sampled (Forbes et al., 1997).

All *P. infestans* isolates from potato landraces grouped in a clade together with known EC-1 isolates, separate from US-1 and *P. andina* isolates (Fig. 1). There is currently some discussion as to whether *P. andina* should be considered a separate species or a hybrid. Although SSR markers are not particularly suitable for species identification, some of the alleles identified in *P. andina* are rare and have never been reported in *P. infestans* isolates. In addition, in *P. andina* there were four alleles at locus Pi4B and null alleles for locus Pi70 which have not been found for other *P. infestans* isolates (Y. Li & T. van der Lee, Plant Research International, Wageningen, The Netherlands, personal communication). This is not a typical pattern for a simple hybridization, but clearly distinguished *P. andina* isolates from *P. infestans* isolates.

This study has identified genetic variation within the *P. infestans* clonal population in Ecuador. The high number of races and their complexity constitute a challenge for late blight management in the country. It is necessary to incorporate new R genes not belonging to the S. demissum group into the National Breeding Programme. Other sources of resistance (Wang *et al.*, 2008; Jacobs *et al.*, 2010; Lokossou *et al.*, 2010) may be useful for potato breeding against P. infestans in Ecuador. However, one promising broad-spectrum resistance gene, Vnt1 (Pel, 2010) cannot be used because the avirulence gene triggering the defense response is not expressed in EC-1 genotypes.

Acknowledgements

We thank the Netherlands Organization for International Cooperation in Higher Education (NUFFIC), the Secretaría Nacional de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT) – Ecuador and the Instituto Nacional Autónomo de Investigaciones Agropecuárias (INIAP) – Ecuador, for financial support. We thank the International Potato Centre (CIP) – Ecuador for sharing lab facilities and technical assistance. We thank Dr. Ronald van den Berg for valuable discussions and producing the dendrogram.

Literature Cited

Abu-El Samen FM, Secor GA, Gudmestad NC, 2003. Variability on virulence among asexual progenies of *Phytophthora infestans*. *Phytopathology* 93, 293-304.

Adler NE, Erselius LJ, Chacon MG, Flier WG, Ordoñez, ME, Kroon LPN, Forbes, GA, 2004. Genetic diversity of *Phytophthora infestans* sensu lato in Ecuador provides insight into the origin of this important plant pathogen. *Phytopathology* 94, 154-162.

Akino S, Baba Y, Kondo N, 2009. Use of microsatellite marker Pi26 for rapid discrimination of five Japanese genotypes of *Phytophthora infestans*. *Journal of General Plant Pathology* 75, 349-352.

Andrivon D, Pilet F, Montarry J, Hafidi M, Cobière R, Achbani EH, Pelle R, Elliseche D, 2007. Adaptation of *Phytophthora infestans* to partial resistance in potato: evidence from French and Moroccan populations. *Phytopathology* 97, 338-343.

Armstrong MR, Whisson SC, Pritchard L, Bos JL, Venter E, Avrova AO, Rehmany AP, Bohme U, Brooks K, Cherevach I, Hamlin N, White B, Fraser A, Lord A, Quail MA, Churcher C, Hall N, Berriman M, Huang S, Kamoun S, Beynon JL, Birch PR, 2005. An ancestral oomycete locus contains late blight avirulence *Avr3a*, enconding a protein that is recognized in host cytoplasm. *Proceedings of the National Academy of Sciences* 102, 7766-7771.

Barquero M, Brenes A, Gómez L, 2005. Complejidad fisiológica de *Phytophthora infestans* en Costa Rica. *Agronomía Costarricense* 29, 21-29.

Black W, Mastenbroek C, Mills WR, Peterson LC, 1953. A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. *Euphytica* 2, 173–8.

Blair JE, Coffey MD, Martin FN, 2012. Species tree estimation for the late blight pathogen, *Phytophthora infestans*, and close relatives. *PLoS ONE* 7(5): e37003. doi:10.1371/journal.pone.0037003.

Blandón-Díaz JU. 2011. *Insights into population structure and epidemiology of Phytophthora infestans from Nicaragua.* Uppsala, Sweden: Swedish University of Agricultural Sciences, PhD Thesis.

Blandón-Díaz JU, Widmark AK, Hannukkala A, Andersson B, Högberg N, Yuen JE. 2012. Phenotypic variation within a clonal lineage of *Phytophthora infestans* infecting both tomato and potato in Nicaragua. *Phytopathology* 102, 323-330.

Cañizares G, Forbes G, 1995. Foliage resistance to *Phytophthora infestans* (Mont.) de Bary in the Ecuadorian national collection of *Solanum phureja* ssp. *phureja* Juz. & Buk. *Potato Research* 38: 3-10.

Cárdenas M, Tabima J, Fry WE, Grünwald NJ, Bernal A, Restrepo S. 2012. Defining species boundaries in the genus *Phytophthora*: the case of *Phytophthora andina* A response to '*Phytophthora andina* sp. nov., a newly identified heterothallic pathogen of solanaceous hosts in the Andean highlands' (Oliva *et al.*, 2010). *Plant Pathology* 61: 215–220.

Caten CE, Jinks JL, 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Canadian Journal of Botany* 46, 329-348.

Chacón MG, 2007. *Variability of Phytophthora infestans sensu lato in the highland tropics of Ecuador.* Zurich: Switzerland: Swiss Federal Institute of Technology Zurich, PhD. Thesis.

Cooke LR, Carlisle DJ, Donaghy C, Quinn M, Perez FM, Deahl KL, 2006. The Northern Ireland *Phytophthora infestans* population 1998–2002 characterized by genotypic and phenotypic markers. *Plant Pathology* 55, 320–330.

Cooke DEL, Cano LM, Raffaele S, Bain RA, Cooke LR, Etherington GJ, Deahl KL, Farrer RA, Gilroy EM, Goss EM, Grünwald NJ, Hein I, MacLean D, McNico JW, Randall E, Oliva RF, Pel MA, Shaw DS, Squires JN, Taylor MC, Vleeshouwers VGAA, Birch PRJ, Lees AK, Kamoun S. (2012) Genome Analyses of an Aggressive and Invasive Lineage of the Irish Potato Famine Pathogen. *PLoS Pathog* 8(10): e1002940. doi:10.1371/journal.ppat.1002940.

Cuesta X, Castillo C, Monteros C, 2005. Biodiversidad de las papas nativas ecuatorianas. In: Monteros C, Cuesta X, Jiménez J, López G, eds. *Las papas nativas en el Ecuador.* Quito, Ecuador: INIAP, 9-10.

Excoffier L, Laval G, Schneider S, 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47-50.

Forbes GA, 1997. *Manual for laboratory work on Phytophthora infestans.* Quito, Ecuador: Centro Internacional de la Papa.

Forbes GA, Escobar XC, Ayala CC, Revelo J, Ordoñez ME, Fry BA, Doucett K, Fry WE, 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. *Phytopathology* 87, 375-380.

Fry WE, Grünwald NJ, Cooke DEL, McLeod A, Forbes GA, Cao K. 2009. Population genetics and population diversity of Phytophthora infestans. In Lamour K & Kamoun S. Oomycete genetics and genomics: Diversity, interactions, and research tools. New Jersey; USA, John Wiley & Sons.

Garry G, Salas A, Forbes GA, Perez W, Santa Cruz M., Nelson RJ, 2005. Host specialization not detected among isolates of the EC-1 lineage of *Phytophthora infestans* attacking wild and cultivated potatoes in Peru. *European Journal of Plant Pathology* 113, 71–81.

Ghimire SR, Hyde KD, Hodgkiss IJ, Liew ECY, 2001. Race diversity and virulence complexity of *Phytophthora infestans* in Nepal. *Potato Research* 44, 253-263.

Grünwald, NJ, Sturbaum AK, Romero Montes, G, Garay Serrano, E, Lozoya-Saldaña H, Fry W E, 2006. Selection for fungicide resistance within a growing season in field populations of *Phytophthora infestans* at the center of origin. *Phytopathology* 96, 1397-1403.

Guo J, van der Lee T, Qu DY, Yao YQ, Gong XF, Liang DL, Xie KY, Wang XW, Govers F, 2009. *Phytophthora infestans* isolates from Northern China show high virulence diversity but low genetic diversity. *Plant Biology* 11, 57-67.

Hermansen A, Hannukkala A, Hafskjold R, Brurberg MB, 2000. Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. *Plant Pathology* 49, 11-22.

Hutcheson K, 1970. A test for comparing diversities based on the Shannon formula. J*ournal of Theoretical Biology* 29, 151-154.

INIAP (Instituto Nacional de Investigaciones Agropecuárias), 1974. Informe Anual de Actividades. Departamento de Fitopatología. Quito, Ecuador: INIAP.

INIAP (Instituto Nacional de Investigaciones Agropecuárias), 1975. Informe Anual de Actividades. Departamento de Fitopatología. Quito, Ecuador: INIAP.

INIAP (Instituto Nacional de Investigaciones Agropecuárias), 1976. Informe Anual de Actividades. Departamento de Fitopatología. Quito, Ecuador: INIAP.

Jacobs MMJ, Vosman B, Vleeshouwers VGAA, Visser RGF, Henken B, van den Berg RG,. 2010. A novel approach to locate *Phytophthora infestans* resistance genes on the potato genetic map. *Theoretical and Applied Genetics* 120, 785-796.

Knapova G, Gisi U, 2002. Phenotypic and genotypic structure of Phytophthora infestans populations on potato and tomato in France and Switzerland. *Plant Pathology* 51, 641–53.

Lees AK, Wattier R, Shaw DS, Sullivan L, Williams, NA, Cooke DE, 2006. Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. *Plant Pathology* 55, 311-319.

Li B, Chen Q, Lv, X, Lan C, Zhao J, Qiu R, Weng Q, 2009. Phenotypic and genotipic characterization of *Phytophthora infestans* isolates from China. *Journal of Phytopathology* 157, 558-567.

Li Y, Govers F, Mendes O, Testa A, Jacobsen E, Huang SW, van der Lee, TAJ, 2010. A new set of highly informative markers for *Phytophthora infestans* population analysis assembled into an efficient multiplex. *Molecular Ecology Resources* 10, 1098-1105.

Li Y, van der Lee T, Evenhuis A et al., 2012a. Population dynamics of *Phytophthora infestans* in the Netherlands reveals expansion and spread of dominant clonal lineages and virulence in sexual offspring. *Genes, Genomes, Genetics* 2, 1529–40.

Li Y, van derLee T, Zhu JH et al., 2012b. Population structure of *Phytophthora infestans* in China – geographic clusters and presence of the EU genotype Blue_13. *Plant Pathology*. Doi: 10.1111/j.1365-3059. 2012.02687.

Lokossou AA, Rietman H, Wang M, Krenek P, van der Schoot H, Henken B, Hoekstra R, Vleeshouwers VGAA, van der Vossen EAG, Visser RGF, Jacobsen E, Vosman B, 2010. Diversity, distribution and evolution of *Solanum bulbocastanum* late blight resistance genes. *Molecular Plant-Microbe Interactions* 23, 1206–1216.

Magurran AE, 1988. *Ecological diversity and its measurement.* Princeton: USA, Princeton University Press.

Malcolmson JF, Black W, 1966. New R-genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica* 15, 199-203.

Montarry J, Corbière R, Lesueur, S, Glais I, Andrivon D, 2006. Does selection by resistant hosts trigger local adaptation in plant-pathogen systems? *Journal of Evolutionary Biology* 19, 522-531.

Montarry J, Hamelin FM, Glais I, Corbière R, Andrivon D, 2010. Fitness costs associated with unnecessary virulence factors and life history traits: evolutionary insights from the potato late blight pathogen *Phytophthora infestans*. *BMC Evolutionary Biology* 10, 283.

Monteros-Altamirano A, 2011. *Potato landraces: description and dinamics in three areas of Ecuador.* Wageningen, The Netherlands: Wageningen University, PhD Thesis.

Monteros A, Vosman B, van der Berg R, 2008. Estudios preliminares para la descripción de papas nativas en tres microcentros de diversidad. In: *Memorias XXIII Congreso Latinoamericano de la Asociación Latinoamericana de la Papa/VI Seminario Latinoamericano de Uso y Comercialización de la Papa*, 2008. Mar del Plata, Argentina: Universidad Nacional de Mar del Plata, 205-206.

Monteros C, Reinoso I,. 2010. Biodiversidad y oportunidades de mercados para las papas nativas ecuatorianas. In: *Memorias XXIV Congreso Latinoamericano de la Asociación Latinoamericana de la Papa.* Cusco, Perú: INIA, 447-448.

Oliva RF. 2009. *Occurrence of sympatric Phytophthora species in the highland of Ecuador.* Zurich, Switzerland: Swiss Federal Institute of Technology Zurich, PhD. Thesis.

Oliva RF, Kroon LPNM, Chacón G, Flier WG, Ristaino JB, Forbes GA, 2010. *Phytophthora andina* sp. nov., a newly identified heterothallic pathogen of solanaceous hosts in the Andean highlands. *Plant pathology* 59, 613-625.

Ordoñez ME, Hohl ME, Velasco JA, Ramon MP, Oyarzún PJ, Smart CD, Fry WE, Forbes GA, Erselius LJ, 2000. A novel population of *Phytophtora*, similar to *P. infestans*, attacks wild *Solanum* species in Ecuador. *Phytopathology* 90, 197-202.

Oyarzún PJ, Pozo A, Ordoñez ME, Doucett K, Forbes GA, 1998. Host specificity of *Phytophthora infestans* on tomato and potato in Ecuador. *Phytopathology* 88, 265-271.

Oyarzún P, Gallegos P, Asaquibay C, Forbes G, Ochoa J, Paucar B, Prado M, Revelo J, Sherwood S, Yumisaca F, 2002. Manejo integrado de plagas y enfermedades. In: Pumisacho M, Sherwood S, eds. *El cultivo de la papa en Ecuador.* Quito, Ecuador: INIAP/CIP, 85-169.

Pel MA, 2010. *Mapping, isolation and characterization of genes responsible for late blight resistance in potato.* Wageningen, The Netherlands: Wageningen University, PhD Thesis.

Revelo J, Garcés S, Andrade J, 1997. Identification of sources of horizontal resistance to *Phytophtora infestans* free of major genes. *CIP Circular* (April), 15-17.

Runno-Paurson E, Fry WE, Remmel T, Mand M., Myers KL, 2010. Phenotypic and genotypic characterization of Estonian isolates of *Phytophthora infestans* in 2004-2007. *Journal of Plant Pathology* 92, 375-384.

Swofford, DL. 2002. PAUP*4.0 Beta, Phylogenetic analysis Using Parsimony (* and other methods). In: Software program Sinauer associates, Sunderland, Massachusetts.

Tello C, 2008. *Identificación de aspectos epidemiológicos relacionados con la expresión de resistencia de la papa (Solanum tuberosum) para las poblaciones de Phytophthora infestans predominantes en tres localidades de la sierra ecuatoriana.* Quito, Ecuador: Universidad Central del Ecuador, BSc Thesis.

Tooley PW, Therrien CD, 1991. Variation in ploidy in *Phytophthora infestans*. In: Lucas JA, Shattock RC, Shaw DS, Cooke LR, eds. *Phytophthora.* Cambridge, UK: Cambridge University Press, 204-217.

van der Lee T, Testa A, van ‘t Klooster J, van der Berg-Velthuis G, Govers F, 2001. Chromosomal deletion in isolates of *Phytophthora infestans* correlates with virulence R3, R10 and R11 potato lines. *Molecular Plant-Microbe Interactions* 14(12): 1444-1452.

van der Lee T, Testa A, Robold A, van 't Klooster J, Govers F, 2004. High-density genetic linkage maps of *Phytophthora infestans* reveal trisomic progeny and chromosomal rearrangements. *Genetics* 167, 1643-1661.

Vargas AM, Quesada Ocampo LM, Céspedes MC, Carreño N, González A, Rojas A, Zuluaga AP, Myers K, Fry WE., Jiménez P, Bernal AJ, Restrepo S. 2009. Characterization of *Phytophthora infestans* populations in Colombia: First report of the A2 mating type. *Phytopathology* 99:82-88.

Vleeshouwers VGAA, Raffaele S, Vossen J, Champouret N, Oliva R, Segretin ME, Rietman H, Cano M, Lokossou AA, Kessel GJT, Pel M, Kamoun S. 2011. Understanding and exploiting late blight resistance in the age of effectors. *Annual Review of Phytopathology* 49, 507-531.

Wang M., Allefs S., van den Berg R.G, Vleeshouwers V., van der Vossen E, Vosman B, 2008. Allele mining in *Solanum*: conserved homologues of *Rpi-blb1* are identified in *Solanum stoloniferum*. *Theoretical and Applied Genetics* 116, 933–943.

Table 1. Diversity of the *Phytophthora infestans* population associated with potato landraces in three provinces of Ecuador. Shown are number of farms from which isolates were collected, the number of isolates, number of races identified, number of SSR profiles as well as Shannon index (H_s) and Evenness (E) based on number of races and SSR profiles.

		Carchi	Chimborazo	Loja	All sites
	No. of farms	8	3	5	16
	No. of isolates	37	20	9	66
Virulence phenotype	Races	27	18	7	49
	H_s	3.15 a	2.86 b	1.89 c	3.78
	E	0.88	0.95	0.86	0.91
SSR Genotype	Genotypes	17	9	8	31
	H_s	2.44 a	1.94 b	2.04 b	3.03
	E	0.68	0.66	0.93	0.72

Values followed by same letter within one line are not significantly different (α=0.05) according to the t-test of Hutcheson for pairwise comparisons.

Table 2. Changes in number of races, Shannon index (H_s) and Evenness (E) of *Phytophthora infestans* populations over the years in Ecuador.

	Year	Provinces		
		Carchi	Chimborazo	Loja
H_s	1990-1993[a]	2.33	2.12	1.55
	2007[b]	2.81	n.e.[d]	n.e.
	2009-2010[c]	3.15	2.86	1.89
E	1990-1993[a]	0.64	0.58	0.45
	2007[b]	0.67	n.e.	n.e.
	2009-2010[c]	0.88	0.95	0.86
Races	1990-1993[a]	14 (n= 39)	14 (n=38)	8 (n= 31)
	2007[b]	27 (n= 68)	n.e.	n.e.
	2009-2010[c]	27 (n= 36)	18 (n= 20)	7 (n= 9)

[a] Calculated from data of Forbes *et al,*. (1997).
[b] Calculated from data of Tello (2008).
[c] Data from this research.
[d] Not evaluated.

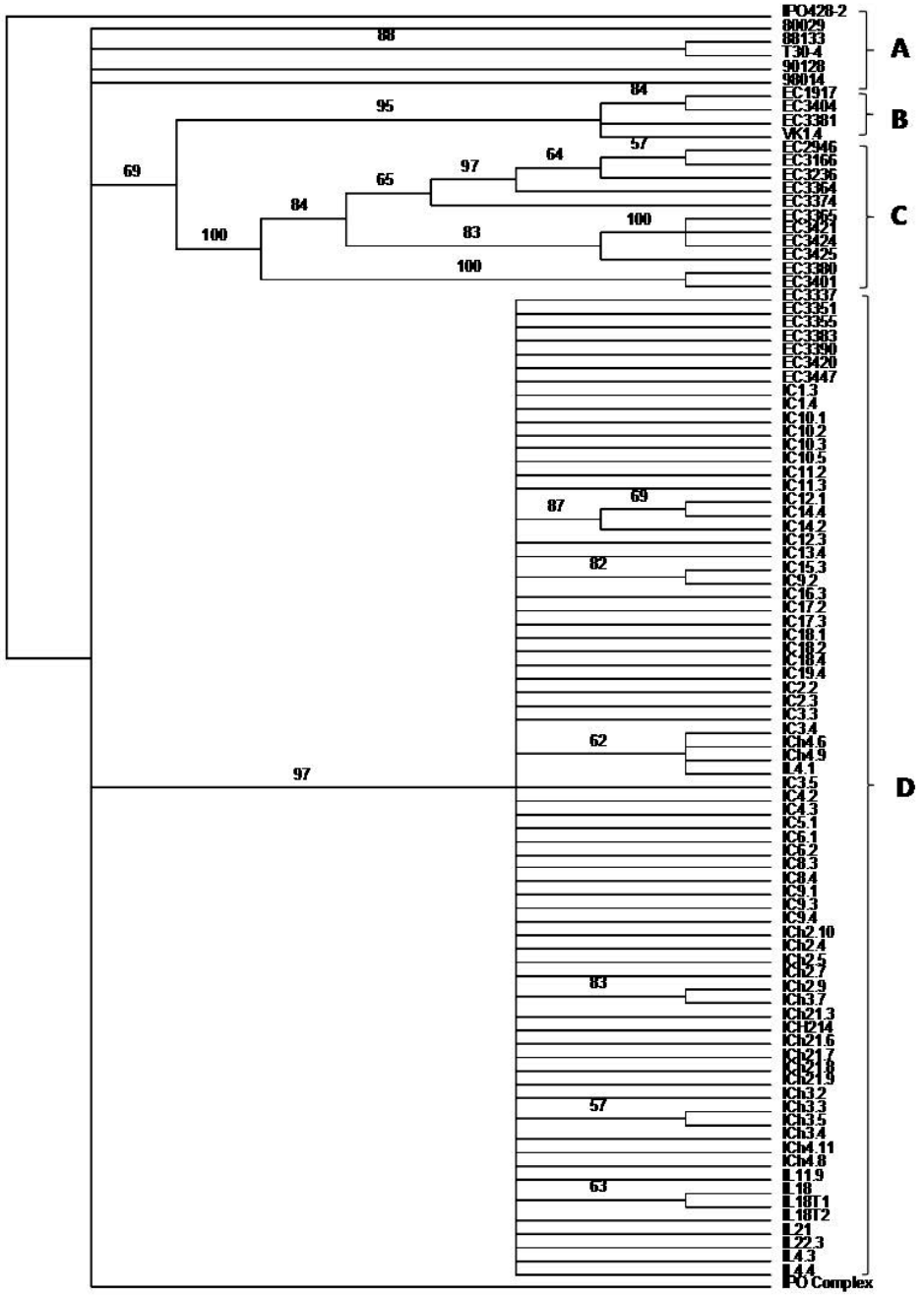


Figure 1. Neighbour-joining tree of the Ecuadorian *Phytophthora infestans* populations associated with potato landraces, based on 12 SSRs. Numbers at the nodes are jackknife values supporting the branches based on 10 000 replicates. (a) European *P. infestans* isolates; (b) *P. infestans* isolates, clonal lineage US-1; (c) *P. andina* isolates; (d) *P. infestans* isolates, clonal lineage EC-1.

Table S1. Isolates associated to potato landraces in Ecuador.

Isolate	Variety Name	Accession Id	Host Ploidy	Race	Place	Province	Altitude (masl)	Latitude	Longitude	Collection Date	SSR Multilocus Genotype
IC1.3	Chaucha Ratona	AXC-1	2x	1,3,4,5,6,7,8,10,11	Ipueran, Julio Andrade, Tulcán	Carchi	3229	N00°40'394"	W77°40'446"	2/Jun/2009	EC-1_001
IC1.4	Chaucha Ratona	AXC-1	2x	1,2,4,6,7,9,10	Ipueran, Julio Andrade, Tulcán	Carchi	3229	N00°40'394"	W77°40'446"	2/Jun/2009	EC-1_003
IC2.2	Amarilla			1,4,5,7,8,10,11	Ipueran, Julio Andrade, Tulcán	Carchi	3229	N00°40'394"	W77°40'446"	2/Jun/2009	EC-1_001
IC2.3	Amarilla			1,3,4,6,7,10,11	Ipueran, Julio Andrade, Tulcán	Carchi	3229	N00°40'394"	W77°40'446"	2/Jun/2009	EC-1_001
IC3.3	Sulipamba	AXC-3	4x	1,4,5,7,10,11	Agua Fuerte, parroquia El Carmelo, Tulcán	Carchi	2985	N00°39'578"	W77°36'560"	2/Jun/2009	EC-1_005
IC3.4	Sulipamba	AXC-3	4x	1,3,4,5,7,8,10,11	Agua Fuerte, parroquia El Carmelo, Tulcán	Carchi	2985	N00°39'578"	W77°36'560"	2/Jun/2009	EC-1_005
IC3.5	Sulipamba	AXC-3	4x	1,4,6,7,8,10,11	Agua Fuerte, parroquia El Carmelo, Tulcán	Carchi	2985	N00°39'578"	W77°36'560"	2/Jun/2009	EC-1_001
IC4.2	Violeta			1,2,3,4,5,6,7,9,10	Agua Fuerte, parroquia El Carmelo, Tulcán	Carchi	2985	N00°39'578"	W77°36'560"	2/Jun/2009	EC-1_017
IC4.3	Violeta			2,3,4,5,7,10	Agua Fuerte, parroquia El Carmelo, Tulcán	Carchi	2985	N00°39'578"	W77°36'560"	2/Jun/2009	EC-1_018
IC5.1	Chaucha Amarilla	AXC-14	2x	1,3,4,5,6,7,10,11	Troya, comunidad Virgen de Fátima, Urbina, Tulcán	Carchi	3362	N00°44'451"	W77°42'129"	2/Jun/2009	EC-1_001
IC6.1	Chaucha Ratona	AXC-1	2x	1,2,3,4,5,7,9,10,11	Troya, comunidad Virgen de Fátima, Urbina, Tulcán	Carchi	3362	N00°44'451"	W77°42'129"	2/Jun/2009	EC-1_001
IC6.2	Chaucha Ratona	AXC-1	2x	1,3,4,7,10	Troya, comunidad Virgen de Fátima, Urbina, Tulcán	Carchi	3362	N00°44'451"	W77°42'129"	2/Jun/2009	EC-1_004
IC8.3	Sabanera	AC-34	4x	1,4,7,9	Chulamuez, Tulcán	Carchi	3316	N00°44'913"	W77°46'389"	2/Jun/2009	EC-1_001
IC8.4	Sabanera	AC-34	4x	1,4,5,7,8,9	Chulamuez, Tulcán	Carchi	3316	N00°44'913"	W77°46'389"	2/Jun/2009	EC-1_004
IC9.1	Chaucha Amarilla			1,3,4,7,10,11	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_020
IC9.2	Chaucha Amarilla			1,4,5,7,8,10,11	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_016
IC9.3	Chaucha Amarilla			1,4,5,7,8,10,11	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_009
IC9.4	Chaucha Amarilla			5,6,7,8,10,11	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_009
IC10.1	Pamba Roja	AXC-17	4x	1,3,4,5,6,8,10,11	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_011
IC10.2	Pamba Roja	AXC-17	4x	1,2,3,4,5,6,7,8,10,11	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_012
IC10.3	Pamba Roja	AXC-17	4x	1,2,3,4,5,7,8,9,10,11	Tufiño Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_001
IC10.5	Pamba Roja	AXC-17	4x	1,2,3,4,5,6,7,8,9,10,11	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_005
IC11.2	Curipamba	AXC-16	4x	2,4,5,6,7,9	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_013

IC11.3	Curipamba	AXC-16	4x	1,2,3,4,5,7,9,10	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_001
IC12.1	Gualcalá	AXC-22	4x	1,3,4,5,6,7,8,10,11	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_006
IC12.3	Gualcalá	AXC-22	4x	1,3,4,5,7,10,11	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_005
IC13.4	Parda Suprema	AXC-21	4x	1,3,4,5,6,7,8,10,11	Tufiño, Tulcán	Carchi	3396	N00°47'806"	W77°52'126"	2/Jun/2009	EC-1_012
IC14.2	Chaucha Amarilla			1,3,4,5,6,7,10,11	Talla/Chapuez Chico, Tulcán	Carchi	3001	N00°46'789"	W77°41'522"	3/Jun/2009	EC-1_031
IC14.4	Chaucha Amarilla			1,3,4,7,8,10,11	Talla/Chapuez Chico, Tulcán	Carchi	3001	N00°46'789"	W77°41'522"	3/Jun/2009	EC-1_006
IC15.3	Rosada			1,4,7,10,11	Talla/Chapuez Chico, Tulcán	Carchi	3001	N00°46'789"	W77°41'522"	3/Jun/2009	EC-1_016
IC16.3	Violeta			1,2,3,4,5,6,7,8,9,10,11	Talla/Chapuez Chico, Tulcán	Carchi	3001	N00°46'789"	W77°41'522"	3/Jun/2009	EC-1_001
IC17.2	Pura Sangre	AXC-13	3x	1,3,4,5,6,7,8,9,10,11	Casa Fría, Julio Andrade, Tulcán	Carchi	3296	N00°42'218"	W77°44'647"	3/Jun/2009	EC-1_021
IC17.3	Pura Sangre	AXC-13	3x	n.e.	Casa Fría, Julio Andrade, Tulcán	Carchi	3296	N00°42'218"	W77°44'647"	3/Jun/2009	EC-1_003
IC18.1	Curipamba			1,3,4,6,7,8,10,11	Casa Fría, Huaca, Montufar	Carchi	3487	N00°43'034"	W77°45'728"	3/Jun/2009	EC-1_001
IC18.2	Curipamba			1,2,3,4,5,6,7,8,910,11	Casa Fría, Huaca, Montufar	Carchi	3487	N00°43'034"	W77°45'728"	3/Jun/2009	EC-1_030
IC18.4	Curipamba			1,3,4,5,6,7,10,11	Casa Fría, Huaca, Montufar	Carchi	3487	N00°43'034"	W77°45'728"	3/Jun/2009	EC-1_004
IC19.4	Rosada			1,3,4,5,6,7,10,11	Casa Fría, Huaca, Montufar	Carchi	3487	N00°43'034"	W77°45'728"	3/Jun/2009	EC-1_001
IL4.1	Chaucha Negra	MPG-26	2x	1,4,7,8,9,10,11	Ciudadela, San Lucas, Loja	Loja	2556	S03°43'261"	W79°14'788"	2/Jul/2009	EC-1_014
IL4.3	Chaucha Negra	MPG-26	2x	1,4,7,8,9,10,11	Ciudadela, San Lucas, Loja	Loja	2556	S03°43'261"	W79°14'788"	2/Jul/2009	EC-1_001
IL4.4	Chaucha Negra	MPG-26	2x	1,4,7,8,9,11	Ciudadela, San Lucas, Loja	Loja	2556	S03°43'261"	W79°14'788"	2/Jul/2009	EC-1_029
IL11.9	Semibolona			1,3,4,5,6,7,8,9,10,11	Huangara, Taquil, Loja	Loja	2402	S03°55'563"	W79°16'281"	13/Aug/2009	EC-1_007
IL22.3	Hualcala	MOPG-011	4x	1,4,7,8,9	Llaco, Tenta, Saraguro	Loja	2552	S03°38'523"	W79°17'334"	30/Sep/2009	EC-1_028
IL18	Negra Carrizo	MOPG-002	4x	1,4,7,8,9,10	Nauchin, Manu, Saraguro	Loja	2840	S 3°32'518"	W79°23'067"	30/Sep/2009	EC-1_025
IL18T1	Negra Carrizo	MOPG-002	4x	1,3,4,5,6,7,9,10,11	Nauchin, Manu, Saraguro	Loja	2840	S 3°32'518"	W79°23'067"	30/Sep/2009	EC-1_026
IL18T2	Negra Carrizo	MOPG-002	4x	1,4,7,9,11	Nauchin, Manu, Saraguro	Loja	2840	S 3°32'518"	W79°23'067"	30/Sep/2009	EC-1_027
IL21	Bodeguera Blanca	MOPG-009	4x	1,3,4,5,6,7,9,10,11	Chorro Blanco, Manu, Saraguro	Loja	2820	S03°31'589"	W79°23'193"	30/Sep/2009	EC-1_007
ICh2.4	Chaucha Blanca	AMA-301		1,3,4,6,7,8,10,11	Utuñag, Penipe	Chimborazo	3032	S01°33'115"	W78°29'379"	1/Dec/2009	EC-1_002
ICh2.5	Chaucha Blanca	AMA-301		1,3,4,5,6,8,9,10,11	Utuñag, Penipe	Chimborazo	3032	S01°33'115"	W78°29'379"	1/Dec/2009	EC-1_001
ICh2.7	Chaucha Blanca	AMA-301		1,3,4,5,7,9,10,11	Utuñag, Penipe	Chimborazo	3032	S01°33'115"	W78°29'379"	1/Dec/2009	EC-1_023
ICh2.9	Chaucha Blanca	AMA-301		1,2,4,5,7,8,9,10,11	Utuñag, Penipe	Chimborazo	3032	S01°33'115"	W78°29'379"	1/Dec/2009	EC-1_008
ICh2.10	Chaucha Blanca	AMA-301		1,3,4,5,6,7,8,9,10,11	Utuñag, Penipe	Chimborazo	3032	S01°33'115"	W78°29'379"	1/Dec/2009	EC-1_022
ICh3.2	Chaucha Negra Pera	AMA-302		1,4,7,8	Utuñag, Penipe	Chimborazo	3032	S01°33'115"	W78°29'379"	1/Dec/2009	EC-1_002
ICh3.3	Chaucha Negra Pera	AMA-302		1,4,6,7,8,9,11	Utuñag, Penipe	Chimborazo	3032	S01°33'115"	W78°29'379"	1/Dec/2009	EC-1_010
ICh3.4	Chaucha Negra	AMA-302		1,4,5,8,9,10,11	Utuñag, Penipe	Chimborazo	3032	S01°33'115"	W78°29'379"	1/Dec/2009	EC-1_002

	Pera										
ICh3.5	Chaucha Negra Pera	AMA-302		1,3,4,5,6,8,9,10,11	Utuñag, Penipe	Chimborazo	3032	S01°33'115"	W78°29'379"	1/Dec/2009	EC-1_010
ICh3.7	Chaucha Negra Pera	AMA-302		1,3,4,5,6,7,8,9,11	Utuñag, Penipe	Chimborazo	3032	S01°33'115"	W78°29'379"	1/Dec/2009	EC-1_008
ICh4.6	Semiuvilla			1,4,5,7,9,10,11	Matus, Penipe	Chimborazo	2679	S01°33'316"	W78°30'429"	1/Dec/2009	EC-1_019
ICh4.8	Semiuvilla			1,3,4,5,6,7,8,9,10	Matus, Penipe	Chimborazo	2679	S01°33'316"	W78°30'429"	1/Dec/2009	EC-1_002
ICh4.9	Semiuvilla			1,2,4,7,9,10,11	Matus, Penipe	Chimborazo	2679	S01°33'316"	W78°30'429"	1/Dec/2009	EC-1_019
ICh4.11	Semiuvilla			1,3,4,7,8,10	Matus, Penipe	Chimborazo	2679	S01°33'316"	W78°30'429"	1/Dec/2009	EC-1_002
ICh21.3	Curipamba			1,4,5,6,7,8,9,10,11	Bacun, Chunchi	Chimborazo	3121	S02°17'294"	W78°53'464"	8/mar/2010	EC-1_003
ICh21.4	Curipamba			1,4,5,6,7,8,9,10,11	Bacun, Chunchi	Chimborazo	3121	S02°17'294"	W78°53'464"	8/mar/2010	EC-1_003
ICh21.6	Curipamba			1,3,4,6,7,9,10,11	Bacun, Chunchi	Chimborazo	3121	S02°17'294"	W78°53'464"	8/mar/2010	EC-1_003
ICh21.7	Curipamba			1,4,5,7,8,9,10,11	Bacun, Chunchi	Chimborazo	3121	S02°17'294"	W78°53'464"	8/mar/2010	EC-1_003
ICh21.8	Curipamba			4,5,7,8,9,10,11	Bacun, Chunchi	Chimborazo	3121	S02°17'294"	W78°53'464"	8/mar/2010	EC-1_024
ICh21.9	Curipamba			1,4,7,9,10,11	Bacun, Chunchi	Chimborazo	3121	S02°17'294"	W78°53'464"	8/mar/2010	EC-1_003

Table S2. Reference isolates used in the genetic study.

Isolate	Host	Species	RG57 RFLP	Mating type	Collection Year	Province	Country
1917	*S. muricatum*				1995	Pichincha	Ecuador
2946					1996		Ecuador
3166	*S. brevifolium*	*P. andina*	EC2	A2	1998	Pichincha	Ecuador
3236	*Solanum spp.*		EC2	A2	1999	Pichincha	Ecuador
3337	*S. paucijugum*				2001	Cotopaxi	Ecuador
3351	*S. tuberosum*	*P. infestans*	EC1	A1	2001	Tungurahua	Ecuador
3355	*S. tuberosum*	*P. infestans*	EC1	A1	2001	Tungurahua	Ecuador
3364	*S. betaceum*	*P. andina*	EC3		2001	Tungurahua	Ecuador
3365	*Anarrhichomenum*	*P. andina*	EC2				Ecuador
3374	*S. brevifolium*				2001	Tungurahua	Ecuador
3380	*S. betaceum*	*P. andina*	EC3		2001	Tungurahua	Ecuador
3381	*S. lycopersicum*	*P. infestans*	US1	A1	2001	Tungurahua	Ecuador
3383	*S. phureja*	*P. infestans*	EC1	A1	2001	Pichincha	Ecuador
3390	*S. solisii*	*P. infestans*	EC1	A1	2001	Tungurahua	Ecuador
3401	*S. betaceum*	*P. andina*	EC3		2001	Tungurahua	Ecuador
3404	*S. caripense*	*P. infestans*	US1	A1	2001	Tungurahua	Ecuador
3420	*S. minutifoliolum*				2001	Tungurahua	Ecuador
3421	*S. muricatum*	*P. andina*	EC2	A2	2001	Tungurahua	Ecuador
3424	*S. muricatum*	*P. andina*	EC2	A2	2001	Tungurahua	Ecuador
3425	*S. brevifolium*	*P. andina*			2001	Tungurahua	Ecuador
3447	*S. phureja*	*P. infestans*	EC1	A1	2001	Cotopaxi	Ecuador
VK1.4	*S. tuberosum*	*P. infestans*	US-1	A1	1958		Netherlands
88133	*S. tuberosum*	*P. infestans*		A2	1988		Netherlands
80029	*S. tuberosum*	*P. infestans*		A1	1980		Netherlands
90128	*S. tuberosum*	*P. infestans*		A2	1990		Netherlands
IPO428-2	*S. tuberosum*	*P. infestans*		A2	1992		Netherlands
T30-4	*S. tuberosum*	*P. infestans*		A1	1998		Netherlands
98014	*S. tuberosum*	*P. infestans*		A1	1998		Netherlands
IPO Complex	*S. tuberosum*	*P. infestans*		A2	1982		Belgium

Table S3. Races of *Phytophthora infestans* associated to potato landraces in Ecuador.

No.	Race	Number of defeated R-genes	Carchi	Chimborazo	Loja	All sites
			No.	No.	No.	No.
1	1,4,7,8	4		1		1
2	1,4,7,9	4	1			1
3	1,3,4,7,10	5	1			1
4	1,4,7,10,11	5	1			1
5	1,4,7,8,9	5			1	1
6	1,4,7,9,11	5			1	1
7	1,3,4,7,10,11	6	1			1
8	1,3,4,7,8,10	6		1		1
9	1,4,5,7,10,11	6	1			1
10	1,4,5,7,8,9	6	1			1
11	1,4,7,8,9,10	6			1	1
12	1,4,7,8,9,11	6			1	1
13	1,4,7,9,10,11	6		1		1
14	2,3,4,5,7,10	6	1			1
15	2,4,5,6,7,9	6	1			1
16	5,6,7,8,10,11	6	1			1
17	1,2,4,6,7,9,10	7	1			1
18	1,2,4,7,9,10,11	7		1		1
19	1,3,4,5,7,10,11	7	1			1
20	1,3,4,6,7,10,11	7	1			1
21	1,3,4,7,8,10,11	7	1			1
22	1,4,5,7,8,10,11	7	3			3
23	1,4,5,7,9,10,11	7		1		1
24	1,4,5,8,9,10,11	7		1		1
25	1,4,6,7,8,10,11	7	1			1
26	1,4,6,7,8,9,11	7		1		1
27	1,4,7,8,9,10,11	7			2	2
28	4,5,7,8,9,10,11	7		1		1
29	1,2,3,4,5,7,9,10	8	1			1
30	1,3,4,5,6,7,10,11	8	4			4
31	1,3,4,5,6,8,10,11	8	1			1
32	1,3,4,5,7,8,10,11	8	1			1
33	1,3,4,5,7,9,10,11	8		1		1
34	1,3,4,6,7,8,10,11	8	1	1		2
35	1,3,4,6,7,9,10,11	8		1		1
36	1,4,5,7,8,9,10,11	8		1		1
37	1,2,3,4,5,6,7,9,10	9	1			1
38	1,2,3,4,5,7,9,10,11	9	1			1
39	1,2,4,5,7,8,9,10,11	9		1		1
40	1,3,4,5,6,7,8,10,11	9	3			3
41	1,3,4,5,6,7,8,9,10	9		1		1
42	1,3,4,5,6,7,8,9,11	9		1		1
43	1,3,4,5,6,7,9,10,11	9			2	2
44	1,3,4,5,6,8,9,10,11	9		2		2
45	1,4,5,6,7,8,9,10,11	9		2		2
46	1,2,3,4,5,6,7,8,10,11	10	1			1
47	1,2,3,4,5,7,8,9,10,11	10	1			1
48	1,3,4,5,6,7,8,9,10,11	10	1	1	1	3
49	1,2,3,4,5,6,7,8,9,10,11	11	3			3
	Total of isolates		36	20	9	65

Table S4. SSR multilocus genotypes of *Phytophthora infestans* isolates associated to potato landraces in Ecuador. Also shown is in which area a particular genotype was found and how often.

	Marker												No Isolates			
Genotype	D13	G11	Pi04	Pi4B	Pi63	Pi70	PinfSSR2	PinfSSR3	PinfSSR4	PinfSSR6	PinfSSR8	PinfSSR11	Carchi	Chimborazo	Loja	Overall
EC-1_001	134	162	170/174	206/214/218	279	191	173/175	268	283/291	242/244	264/266	330/356	12	1	1	14
EC-1_002	134	162	170/174	206/214/218	279	191	173/175	268	283/291/293	242/244	264/266	330/356		5		5
EC-1_003	132/134	162	170/174	206/214/218	279	191	173/175	268	283/291/293	242/244	264/266	330/356	2	5		7
EC-1_004	132/134	162	170/174	206/214/218	279	191	173/175	266/268	283/291	242/244	264/266	330/356	3			3
EC-1_005	132/134	162	170/174	206/214/218	279	191	173/175	268	283/291	242/244	264/266	330/356	3			3
EC-1_006	134	162	170/174	206/218/222	279	191	173/175	264/268	283/291	242/244	264/266	330/356	2			2
EC-1_007	134	162	170/174	206/214/218	279	191	173/175	268	283/291/295	242/244	264/266	330/356			2	2
EC-1_008	134	162	170/174	206/214/218	279	191	173/176	268	283/291	242/244	264/266	330/356		2		2
EC-1_009	134/136	162	170/174	206/214/218	279	191	173/175	268	283/291/295	242/244	264/266	330/356	2			2
EC-1_010	134	162	170/174	206/214/218	279	191	173/175	268	283/293	242/244	264/266	330/356		2		2
EC-1_011	134	162	170/174	214/218	279	191	173/175	268	283/291	242/244	264/266	356	1			1
EC-1_012	134/136	162	170/174	206/214/218	279	191	173/175	268	283/291	242/244	264/266	330/356	2			2
EC-1_013	132/134	162	170/174	206/214/218	279	191	173/175	268	283/291	244	264/266	330/356	1			1
EC-1_014	134/138	162	170/174	206/214/218	279	191	173/175	268	283/291/295	242/244	264/266	330/356			1	1
EC-1_015	134/138	162	170/174	206/214/218	279	191	173/175	268	283/291	242/244	264/266	330/356	1			1
EC-1_016	134	162/166	170/174	206/214/218	279	191	173/175	268	283/289/291	242/244	264/266	330/356	2			2
EC-1_017	134	162	170/174	206/214/218	279	191	173/175	268	283/291	242/244	264/266	356	1			1
EC-1_018	134	162	170/174	206/214/218	279	191	173/175	268	283/291/293	242/244	264/266	356	1			1
EC-1_019	134/138	162	170/174	206/214/218	279	191	173/175	268	283/291/293	242/244	264/266	330/356		2		2

EC-1_020	134	162	170/174	206/214/218	279	191	173	266/268	283/291/293	242/244	264/266	330/356	1			1
EC-1_021	132/134	162	170/174	206/214/218	279	191	173/175	266/268	283/289/293	242/244	264/266	330/356	1			1
EC-1_022	134	158/162	170/174	206/214/218	279	191	173/175	266/268	283/293	242/244	264/266	330/356		1		1
EC-1_023	130/134	162	170/174	206/214/218	279	191	173/175	268	283/291/293	244	264/266	330/356		1		1
EC-1_024	132/134	162	170/174	206/214/218	279	191	173/175	266/268	283/291/295	242/244	264/266	330/356		1		1
EC-1_025	134/142	162	170/174	206/214/218	279	191	173/175	268	283/291/295	242/244	264/266	330/356			1	1
EC-1_026	134/142	162	170/174	206/214/218	279	191	173/175	266/268	283/291/295	242/244	264/266	330/356			1	1
EC-1_027	134	162	170/174	206/214/218	279	191	173/175	266/268	283/291/295	242/244	264/266	330/356			1	1
EC-1_028	134	162	170/174	214/218	279	191	173	268	283/291/293	242/244	264/266	330/356			1	1
EC-1_029	134	160/162	170/174	206/214/218	279	191	173/175	268	283/291	242/244	264/266	330/356			1	1
EC-1_030	134	162	170/174	206/214	279	191	173/175	268	283/291	242/244	264/266	330/356	1			1
EC-1_031	134	162	170/174	206/218/222	279	191	173/175	264/268	283/291	244	264/266	330/356	1			1

Table S5. SSR genotypes observed in reference isolates.

		D13	G11	Pi04	Pi4B	Pi63	Pi70	PinfSSR2	PinfSSR3	PinfSSR4	PinfSSR6	PinfSSR8	PinfSSR11
EC3337	EC-1	134/136	162	170/174	206/214/218	279	191	173/175	264/268	283/291	242/244	264/266	330/356
EC3351	EC-1	134	162	170/174	206/214/218	279	191	173/175	268	283/291/295	242/244	264/266	330/356
EC3355	EC-1	134	162	170/174	206/216/218	279	191	173/175	268	283/291/293	242/244	264/266	330/356
EC3383	EC-1	134	162	170/174	206/214/218	279	191	173/175	268	283/291/295	242/244	264/266	330/356
EC3390	EC-1	134	162/174	170/174	206/214/218	279	191	173/175	268	283/291/295	242/244	264/266	330/356
EC3420	EC-1	134	162	170/174	206/214/218	279	191	173/175	268	283/291	242/244	264/266	330/356
EC3447	EC-1	134	162	170/174	206/214/218	279	191	173/175	268	283/291/295	242/244	264/266	330/356
EC3404	US-1	134/136	158/168	170/174	214/218	270/273/279	188/191	173/177	266	287/289/299	244	266	340/356
EC3381	US-1	136/140	158	174	214/222	270/273/279	188/191	173/177	266	289/297	244	266	340/356
EC1917	US-1	134	158/168	170/174	214/218	270/273/279	188/191	173	266	287/289/299	244	266	340/356
VK1.4	US-1	138	158/162	170/174	214/218	270/273/279	188/191	173/177	266	287/289	244	266	340/356
428.2		134	162	170/174	214/218	273/279	191/194	173	258/268	293	242	260/266	330/356
80029		116/134		172	214	279	191	173	268	283/293	244	260/266	340
88133		152/154	162/168	174	214	279	191/194	173/175	268/270	287/297	242/244	260/264	330/340
90128			162	172	214/218	273/279	191	173/175	268	293	242/244	260/266	340/356
98014		134	160/162	170/174	218	270/273/279	191/194	173	258/268	287/289/291	242/244	260/264/266	340/356
IPO complex			148/162	172	218	279	191/194	173	268	287/293	244	260/266	340
T30-4		116	162	174	214	279	191	173/175	268/270	283/287	244	260/264	340
EC2946	*P. andina*	147/149	137	174	210/248/268	273		173/176	266	281/287	240/244	260/266	340
EC3166	*P. andina*	147/149	130/140	174	210/248/264/268	273		173/176	264/266	281/287	240/244	260/266	340

EC3236	*P. andina*		130/140	174	210/248/268	273	191	173/176	266	281/287	240/244	260/266	340
EC3364	*P. andina*	142	137	174	210/248/268	273	191	173/176	266	281/287	240/244	260/266	340
EC3365	*P. andina*	105	135	170/174	210/244/297	273		173/176	266	281	240	260/266	356
EC3374	*P. andina*	105	135/166	174	210/248/264/284	273	191	173/176	266	281/287	240/244	260/266	356
EC3380	*P. andina*	105	137/160	174	214/244/256/270	273	191	173/176	266	280	240/244	260/266	340
EC3401	*P. andina*	105	137/172	174	214/244/256/270	273	191	173/176	264	280	240/244	260/266	340
EC3421	*P. andina*	105	135	170/174	210/244/297	273		173/176	266	281	240	260/266	356
EC3424	*P. andina*	105	135	170/174	210/244/297	273		173/176	266	281	240	260/266	356
EC3425	*P. andina*	108	135	170/174	210/248/289	273	194	173/176	266	281	240	258/260	356

Figure S1. Electropherograms of the fluorescent amplification products for locus Pi4B in isolates of *P. andina*. A: EC3365; B: EC3380.

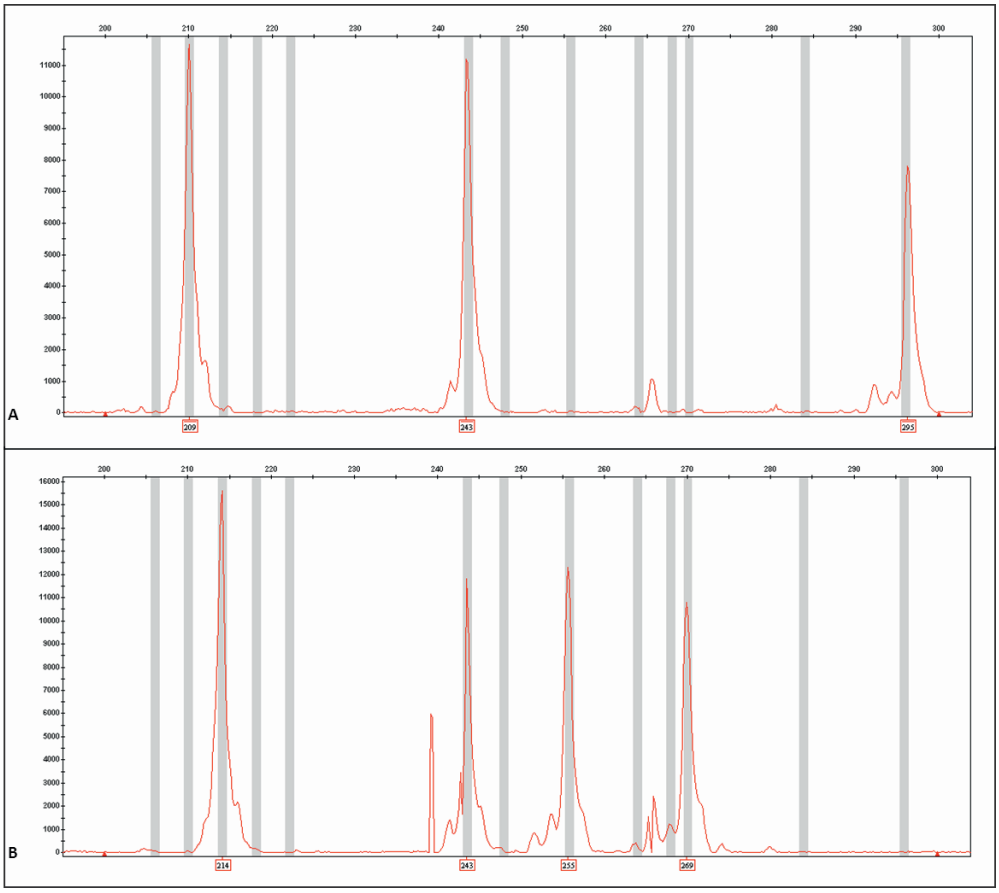
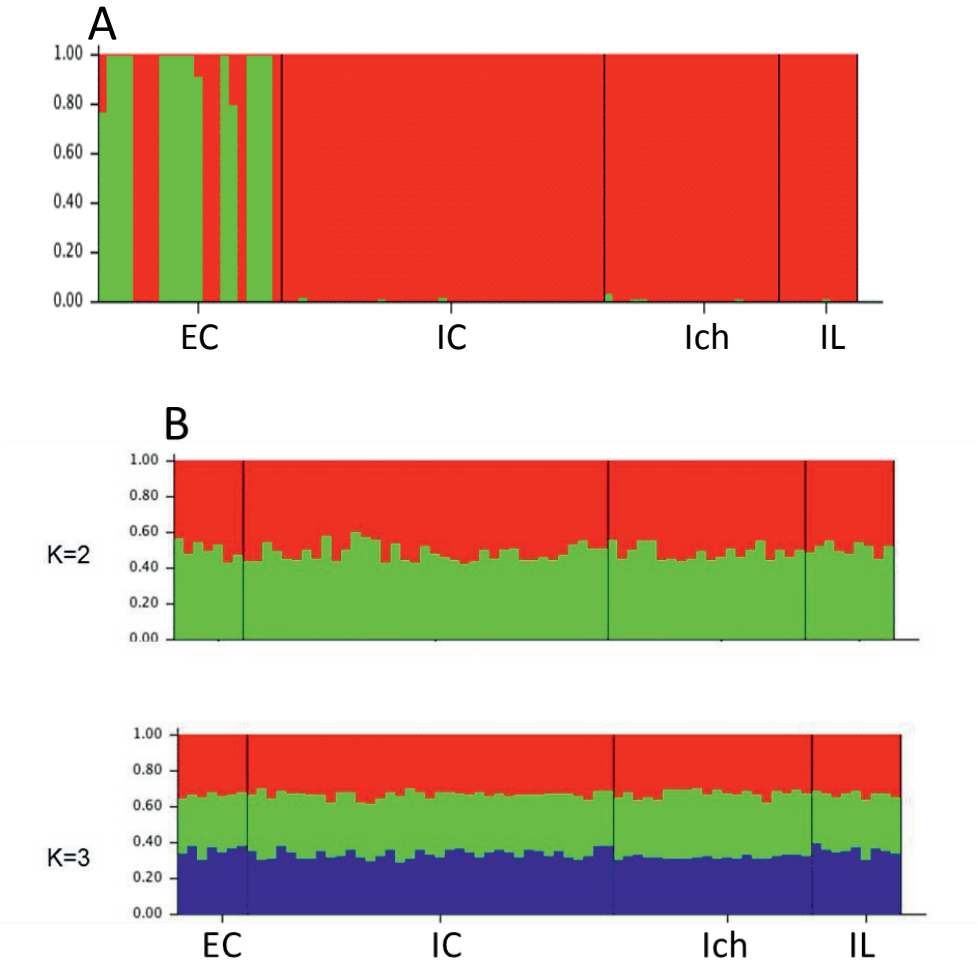


Figure S2. STRUCTURE analysis of the Ecuadorian *P. infestans* populations associated to potato landraces based on 12 SSRs.

A: The whole Ecuadorian population. All material originating from Ecuador described in Supplementary tables 1 and 2 is included. Summary of results for STRUCTURE analysis at K=2 showing the proportion of all isolates from the three sampling regions. IC= Carchi, Ich= Chimborazo and IL=Loja. EC= Ecuadorian reference material from Supplementary table 2. Red color = *P. infestans;* green color = *P. andina.*

B: Structure of the clonal lineage. Material included all *P. infestans* isolates Supplementary tables 1 + the *P. infestans* reference material from Supplementary table 2. The STRUCTURE output at (K=2 and K=3) is presented.



Chapter 4

Exploring the reaction of Ecuadorian potato landraces to late blight

Ricardo A. Delgado[1,2,3], Richard G.F. Visser[2]

[1] Instituto Nacional Autónomo de Investigaciones Agropecuárias (INIAP), Estación Experimental Santa Catalina, Panamericana Sur km 1, Quito, Ecuador.

[2] Plant Breeding, Wageningen University and Research, P.O. Box 386, 6700 AJ, Wageningen, The Netherlands.

[3] Graduate school Experimental Plant Sciences, Wageningen University

Abstract

Late blight is the most devastating disease of potatoes in Ecuador, which makes breeding for disease resistance a necessary and continuous effort. There are more than 400 potato landraces in Ecuador, which belong mainly to *Solanum phureja* and *S. andigena* species. The goal of this research was to evaluate the potential of a selection of potato landraces as a source for late blight resistance. Two field trials were set in San Pedro de Huaca, Carchi province. The first consisted of 52 Ecuadorian landraces, two landraces from Perú and one from Colombia and four Ecuadorian commercial varieties. The second one consisted of 15 landraces. Both trials were planted in a random block design with three replications. Disease severity was recorded under natural infection and the Area Under Disease progress Curve (AUDPC) was calculated. An aggressiveness test was conducted in the lab with three *Phytophthora infestans* isolates. These were inoculated on detached leaflets of a set of five selected landraces and two commercial varieties. After six days inoculated leaflets were photographed and lesion size measured with ImageJ software and the percentage of leaflet area covered by the disease calculated. In the field trials, the landraces Santa Rosa Amarilla, Uva, Coneja Blanca and Botella were resistant to late blight and grouped with INIAP Estela, Fripapa and Natividad varieties. In the aggressiveness test, it was observed that the ranking of the genotypes varied depending on the isolate. There is quantitative resistance in Ecuadorian potato landraces. The resistance detected seems to be of a similar level as that of improved varieties released in the country. Detached leaf assays may give an indication on the reaction of the genotypes, but field evaluation is necessary to confirm the trait. For future breeding, the resistant accessions observed can be used in combination with other quantitative resistant genotypes and/or carriers of major resistance genes to breed for (quantitative) resistant varieties.

Keywords: breeding, resistance, *Phytophthora infestans*

Introduction

Late blight is the most important disease of potatoes worldwide as well as in Ecuador (Oyarzún *et al.*, 2002). It is caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. The yield losses due to late blight disease can be enormous (up to 100%) depending on the cultivar's level of resistance and weather conditions (Morales *et al.*, 1995). The use of varieties with resistance to the disease is one of the alternatives for reducing the damage induced by the pathogen. Breeding efforts to obtain more resistant varieties are continuously being carried out, but it can take several years until a new variety is ready for release (Cuesta, 2011; Cuesta et al, 2015). An additional difficulty is caused by the population of *P. infestans*, which consists of a clonal lineage in Ecuador, named EC-1, but despite the lack of sexual reproduction, it is composed of highly complex races (Forbes *et al.*, 1997, Tello, 2008, Delgado & Vosman, 2010, Chapter 3, Delgado et al, 2013) and capable to overcome major resistance genes incorporated in improved varieties (Revelo *et al*., 1997; Oyarzún *et al*., 2002).

Two types of resistance are known: vertical resistance or horizontal resistance. Vertical resistance is based on major genes (R genes) and is associated with hypersensitive response leading to cell death and, preventing further colonization of the host tissue. Horizontal resistance, also known as quantitative or field resistance, is based on multiple genes that each have a relatively small effect and, similarly effective against a wide spectrum of races of *P. infestans* and stable (Acquaa, 2007; Brown & Caligari, 2008; Sliwka & Zimnoch-Guzowska, 2013).

There is a continuous need for identifying sources for resistance to the disease in the country. According to Camacho et al (2006), "A landrace is a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems". The local potato landraces could be a possible source for this character. These landraces are the result of selection and conservation carried out by the farmers in the highlands (Cuesta *et al.*, 2005). These potatoes have been cultivated under conditions different from the commercial varieties, mainly on a small scale with low use of pesticides (Cuesta *et al.*, 2005, Monteros & Reinoso, 2010). It has been estimated that there are over 400 landraces in the country (Cuesta *et al.*, 2005). The landraces in Ecuador are mainly from the species *S. andigena*, *S. phureja* and *S. chaucha* (Monteros-Altamirano, 2011).

The potato landraces have been reported as sources of quantitative resistance in several countries. This type of resistance is attributed to the effect of several genes leading to a partial resistance to all the races of the pathogen and, considered stable and durable (Slidwa & Zimnoch-Guzowska, 2013). In Colombia, clones mainly from *S. phureja* had been reported as sources of quantitative resistance (Thurston et al, 1962; Escallon et al., 2005; Mosquera, 2007) and *S. andigena* too (Thurston et al, 1962). Meanwhile, in Bolivia, accessions from *S. andigena* and *S. phureja* showed resistance to late blight as well (Gabriel et al., 2007; Gabriel et al., 2013). Additionally, in Perú, Perez et al (2014) reported different species of landraces with resistance to the disease equal or less susceptible than the resistant control, including several accessions of *S. andigena*, one of them from Ecuador. Finally, potato landraces belonging to *S. phureja* have been reported as sources of quantitative resistance in Ecuador (Cañizares & Forbes, 1995, Revelo *et al.*, 1997a, Garofalo, 2005).

The use of laboratory techniques for screening late blight resistance, like detached leaf assays have been proposed for the characterization of germplasm (Vleeshouwers et al 1999). Some researchers reported low correlation among field behavior and detached leaf assays when screening potatoes (Filippov et al, 2004, Rogozina et al, 2010, Sharma et al, 2013).

The purpose of this research was to explore the potential of a set of Ecuadorian potato landraces as sources for late blight resistance under field conditions and the effect of different isolates on the reaction to the disease.

Materials and methods

Field Trials

For the evaluation of late blight resistance of a collection of genotypes maintained by the National Potato Program of the Instituto Nacional Autónomo de Investigaciones Agropecuárias (INIAP), two field trials were conducted in San Pedro de Huaca, Province of Carchi, Ecuador. The farm is located 77°43'35" longitude, 00°38'29" N latitude, 2952 masl with 1100 mm of average rainfall/year, 76% average relative humidity and 10 °C average temperature/year. This location was chosen since several complex races had been observed there (Tello, 2008). Two trials were necessary because of the size of the field and the accompanying workload, as well as because of the availability of planting material. The first trial consisted in 52 Ecuadorian landraces plus three landraces from neighboring countries (Natin Suito/CIP702464 from Colombia, Amarilla/CIP704481 and Puca Huayro/CIP701524 both from Peru) and 4 commercial varieties (INIAP-Estela, INIAP-Fripapa, INIAP-Natividad and Superchola) as checks. The second trial consisted of 15 Ecuadorian potato landraces. Both trials included a susceptible control, the landrace named 'Uvilla' (Revelo *et al.*, 1997b), as well as the landraces Bolona, Puña and Coneja Negra to be able to compare results between the two trials. Both trials were carried out in a Random Block Design (RBD) with 4 replicates in the first trial and 5 in the second, each replicate consisted of 10 tubers as recommended by CIP (2006). Natural infection by the pathogen was allowed. Disease severity was recorded as percentage of necrotic tissue. The evaluation started when the first symptoms were observed and they were scored weekly. The late blight severity values were used to calculate the Area Under the Disease Progress Curve (AUDPC) according to Shanner & Finney (1977):

$$AUDPC = \sum_{i=1}^{n} [(Y_{i+n1} + Y_i)/2] [X_{i+1} - X_i]$$

in which Y_i = late blight severity (per plot) at the i*th* observation, X_i = time (days) at the i*th* observation, and n = total number of observations.

Additionally, the cultivars were classified based on their susceptibility to late blight according on their average AUDPC values: highly resistant (< 250), resistant (251-550), moderately

resistant (551-650), susceptible (651-1200) and highly susceptible (>1200) (Gopal & Singh, 2003).

Aggressiveness test

Three isolates of *P. infestans* obtained from potato landraces were used. They were inoculated on a set of five potato landraces plus the commercial varieties Superchola and Fripapa. The experiment was set as a Complete Random Design with the potato varieties as treatments. From each potato genotype, six leaflets were taken and placed in inverted Petri dishes containing Water-Agar. On the abaxial surface of each leaflet, one 20 µL drop containing 25 x 10^3 sporangia/mL was placed at one side of the mid vein. The inoculated leaves were placed in climate chamber at 16 °C for six days with 12 hours of photoperiod (Forbes, 1997). Thereafter, each leaflet was photographed with a digital camera. Using the software ImageJ (Abràmoff *et al.*, 2004), the size of the lesion and area of leaflet was measured with the program as described by Delgado & Tello (2010) (Figure 1). The percentage of area diseased was calculated for each leaflet.

Statistical Analysis

For the field trial, the Analysis of Variance of AUDPC values was performed. In the case of the laboratory trial for the Analysis of Variance, the square root of the percentage of diseased was used. Scott & Knott test was used for mean analysis in all the cases (Gates & Bilbro, 1978).

Results

The severity observed in field conditions for both trials showed that majority of them behaved as susceptible to late blight with few ones with lower values at the end of the evaluation (Fig. 2 and 3). For the first field trial, there were significant differences in the late blight resistance of the potato genotypes evaluated ($F=6.78$, d.f.=58, $p<0.0001$). The Scott & Knott test allowed distinguishing the genotypes into three groups. The resistant group, which had the lowest average values of AUDPC but never an absolute resistance, was formed by seven genotypes. They included the commercial varieties INIAP-Estela, Fripapa and Natividad together with the landraces Santa Rosa Amarilla, Uva, Coneja Blanca and Botella. The rest of the genotypes were intermediate (27) or susceptible (25) to late blight (Table 2). In the second field trial, there were significant differences in the reaction to late blight among the landraces evaluated ($F=16.82$, d.f.=14, $p<0.0001$). Three groups of landraces were formed according to their reaction to late blight. Suscaleña Amarilla and Suscaleña Negra were the most resistant ones. Seven had an intermediate reaction and six were susceptible. When analyzed the reaction to the disease according AUDPC values (Gopal & Singh, 2001), just two in the field trial (Santa Rosa Amarilla and Uva) and two in the second trial (Suscaleña Amarilla and Suscaleña Negra) were moderately resistant and resistant, respectively (Table 1 and 2).

In the aggressiveness test, inoculated genotypes were separated in two groups for isolates IC6.2 and ICH21.9, one resistant and one susceptible. For IL4.1, the varieties formed three groups; resistant, susceptible and intermediate. The genotype AC-37, grouped with the susceptible group when inoculated with ICh21.9, but behaved as resistant with IC6.2 and

intermediate with IL4.1. The variety INIAP-Fripapa, grouped with the resistant ones when inoculated with ICh21.9 and IL4.1, but susceptible with IC6.2 (Table 3).

Discussion

In the first field trial, it was observed that few landraces were resistant to late blight. Santa Rosa Amarilla, Uva, Coneja Blanca and Botella were grouped together with the commercial varieties INIAP-Estela, INIAP-Fripapa and INIAP-Natividad. These varieties have been selected for resistance to late blight (Cuesta el al, 2002, Cuesta *et al.*, 2008). INIAP-Fripapa is supposed to have major resistance genes (Revelo *et al.*, 1997). Superchola and Uvilla appeared in the intermediate group; nevertheless both had been described as susceptible to late blight (Revelo *et al.*, 1997). The rest of the landraces varied from moderate to highly susceptible. The landraces from the other Andean countries behave as part of the intermediate group. In the second field trial, the same tendency was observed with two resistant landraces (Suscaleña Amarilla and Suscaleña Negra) and the rest being moderate to highly susceptible.

In both trials, it was observed that most of the genotypes showed susceptibility to late blight which agrees with previous observations on landraces in Bolivia, Colombia, Peru and Ecuador. Those studies evidenced a wide variation in the reaction to late blight from high susceptibility to some resistance with a small number of resistant genotypes (Thurston et al, 1962, Cañizares & Forbes, 1995, Revelo *et al.*, 1997, Gabriel *et al.*, 2007, Gabriel et al, 2013, Perez et al, 2014).

In the aggressiveness test, it was observed that the ranking of the genotypes varied depending on the isolate. The variety Fripapa which behaved as resistant in the field trial, varied in the detached leaf assay, depending on the used isolate. Gabriel *et al.*, (2007) reported no correlation among lesion size on detached leaves and AUDPC values from a field test among 17 Bolivian potato landraces. One of these genotypes tested in the field trial -Natin Suito- which was susceptible to late blight, was indicated as resistant in a detached leaf assay in Bolivia (Gabriel *et al.*, 2008). Rogozina *et al.*, (2010) observed low correlation among laboratory inoculation tests and field trials. They even observed that genotypes from *Solanum* species resistant in the field were infected in detached leaf tests. Similarly, Filippov et al (2004), reported that resistance of varieties varied according to the origin of the isolates used in detached leaf assay. Sharma et al (2013), found a low correlation among detached leaf assay and field reaction. A possible explanation is given by the different conditions of the resistance tests. While in the field trial the genotypes are exposed to a population of the pathogen, in laboratory tests a single isolate is used. In our country there exists a high variability within the *P. infestans* populations which are highly complex (Forbes *et al.*, 1997, Tello, 2008, Chapter 3; Delgado *et al.*, 2013) and capable to overcome major resistance genes (Revelo *et al.*, 1997).

From these experiments it is clear that there is quantitative resistance in Ecuadorian potato landraces. The resistance detected is not very strong but seems to be similar to that of some

improved varieties released in the country. Detached leaf assays may give an indication on the reaction of the genotypes, but field evaluation is necessary to confirm the usable level of resistance.

The potato landraces evaluated in this study may have other valuable traits other than late blight resistance which may explain its survival until nowadays.

It is clear that for future breeding, the quantitative resistant accessions observed have to be used in combination with other (quantitative) resistance types to come to varieties which are highly resistant to late blight.

Acknowledgments

To the Netherlands Organization for International Cooperation in Higher Education (NUFFIC), the Secretaria Nacional de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT), and the Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP) for the financial support. To the INIAP's staff in Carchi for their assistance with the field trials. To the International Potato Center (CIP) – Ecuador for sharing lab facilities and expertise.

Literature cited

Abràmoff, M.D., Magalhães, P.J. and Ram, S.J. Image Processing with ImageJ. Biophotonics International, 11(7):36–42, 2004.

Acquaah G. 2007. Principles of Plant Genetics and Breeding. Blackwell Publishing. 569 p.

Brown J., Caligari P. 2008. An introduction to plant breeding. Blackwell Publishing: 209 p.

Camacho C., Maxted N., Scholten M., Ford-Lloyd B. 2006. Defining and identifying crop landraces. Plant Genetic Resources 3 (3): 373-384.

Cañizares, C.A., Forbes, G.A. 1995. Foliage resistance to Phytophthora infestans (Mont.) de Bary in the Ecuadorian national collection of Solanum phureja ssp. phureja Juz. & Buk Potato Research 38: 3-10.

Cuesta, X., Andrade, H., Bastidas, O., Quevedo, R., Sherwood, S. 2002. Botanica y mejoramiento genetico. . *In* Pumisacho, M.; Sherwood, S. El cultivo de la papa en Ecuador. Quito, INIAP/CIP. Pp. 34-49.

Cuesta, X.; Castillo, C.; Monteros, C. 2005. Biodiversidad de las papas nativas ecuatorianas. *In* Monteros, C.; Cuesta, X.; Jiménez, J.; López, G. (Eds.). Las papas nativas en el Ecuador. Quito, Ecuador, INIAP. Pp. 9-10.

Cuesta, X., Rivadeneira, J., Carrera, E., Monar, C., Lucero, H., Suquillo, J., Sevillano, C., Reinoso, I. 2008. Tres nuevas variedades de papa con resistencia y calidad. *In* III Congreso Nacional de la Papa.

Delgado, R.; Tello, C. 2010. Procedimiento para la estimación del área de la lesión mediante ImageJ. *In* Taller en Métodos aplicados en evaluaciones y análisis en la mejora genética de la papa. March 22-26, 2010. INIAP-CIP-NEIKER, Quito, Ecuador.

Delgado, R. A., Monteros-Altamirano, A. R., Li, Y., Visser, R. G. F., van der Lee, T. A. J. and Vosman, B. 2013. Large subclonal variation in Phytophthora infestans populations associated with Ecuadorian potato landraces. Plant Pathology, 62: 1081–1088. doi: 10.1111/ppa.12039

Escallon, R., Ramírez, M., Ñústez, C.E. 2005. Evaluación del potencial de rendimiento y de la resistencia a *Phytophthora infestans* (Mont. de Bary) en la colección de papas redondas amarillas de la especie *Solanum phureja* (Juz. *et* Buk.). Agronomía Colombiana 23 (1): 35-41.

Filippov, A.V., Gurevich, B.I., Kozlovsky, B.E., Kuznetsova, M.A., Rogozhin, A.N., Spiglazova, S.Y., Smetanina, T.I., Smirnov, A.N. 2004. A rapid method for evaluation of partial potato resistance to late blight and of aggressiveness of *Phytophthora infestans* isolates originating from different regions. Plant Breeding and Seed Science 50, 29-41.

Forbes, G.A. 1997. Manual for laboratory work on *Phytophthora infestans.* Centro Internacional de la Papa, Quito, Ecuador. 33p.

Forbes GA, Escobar XC, Ayala CC, Revelo J, Ordoñez ME, Fry BA, Doucett K, Fry WE. 1997. Population Genetic Structure of Phytophthora infestans in Ecuador. Phytopathology. 87(4):375-80.

Gabriel, J., Coca, A., Plata, G., Parlevliet, J.E. 2007. Characterization of the resistance to *Phytophthora infestans* in local potato cultivars in Bolivia. Euphytica 153: 321-328.

Gabriel, J., Coca, J., Angulo, A., Franco, J. Plata, G. 2008. Germplasma nativo de papa evaluado por su reaccion de resistencia y/o susceptibilidad a factores bioticos y abioticos. *In* III Congreso Iberoamericano de Investigacion y Desarrollo en Patata. Pp.53-57.

Gabriel J, Plata G, Cadima X, Franco J. 2013. *Solanum phureja* Juz et Burk.: Valuable source of genetic resistance to potato late blight (*Phytophthora infestans* (Mont.) de Bary). Revista Latinoamericana de la Papa 17 (2), 131-142.

Gates, C.E., Bilbro, J.D. 1978. Illustration of a cluster analysis method for mean separation. Agronomy Journal 70: 462–465.

Garofalo, J. 2005. Evaluación de la aptitud combinatoria general y específica en 21 progenies de papa Solanum phureja para resistencia a tizón tardío Phytophthora infestans. Tesis Ing. Agrónomo. Quito, Universidad Central del Ecuador. 123p.

Gopal, J., Singh, B.P. 2003. Screening potatoes for resistance to late blight (Phytophthora infestans) under field conditions. Potato Res 46: 47-56.

International Potato Center (CIP). 2006. Procedures for standrd evaluation trials of advanced potato clones. An international Cooperator's Guide. Lima, Perú: CIP.

Morales, R., Revelo, J., Suarez, G., Forbes, G., Muñoz, F. 1995. Relación entre el ataque de *Phytophthora infestans* y la producción en el cultivo de la papa (*Solanum tuberosum*). In Memorias XVII Reunión de la Asociación Latinoamericana de la Papa. Mérida, Venezuela.

Monteros-Altamirano A, 2011. *Potato landraces: description and dynamics in three areas of Ecuador.* Wageningen, The Netherlands: Wageningen University, PhD Thesis.

Monteros, A.; Vosman, B.; Van Der Berg, R. 2008. Estudios preliminares para la descripción de papas nativas en tres microcentros de diversidad. *In* Memorias XXIII Congreso Latinoamericano de la Asociación Latinoamericana de la Papa/VI Seminario Latinoamericano de Uso y Comercialización de la Papa. 30 de noviembre al 6 de diciembre de 2008. Mar del Plata, Universidad Nacional de Mar del Plata. Pp. 205-206.

Monteros, C.; Reinoso, I. 2010. Biodiversidad y oportunidades de mercados para las papas nativas ecuatorianas. *In* Memorias XXIV Congreso Latinoamericano de la Asociación Latinoamericana de la Papa. Cusco, Perú, INIA. Pp. 447-448.

Oyarzún, P.J., Taipe, J.A., Forbes, G.A. 2002. *Phytophtora infestans* su actividad y particularidades en el Ecuador. Perfil del Pais. *In* Fernandez-Northcote, E.N. (ed.) Memorias del Taller Internacional 'Complementando la resistencia al tizon (*Phytophtora infestans*) en los Andes'. February 13-16, 2001. Cochabamba, Bolivia. CIP, Lima. Pp. 17-27.

Oyarzún, P.; Gallegos, P.; Asaquibay, C.; Forbes, G.; Ochoa, J.; Paucar, B.; Prado, M.; Revelo, J.; Sherwood, S.; Yumisaca, F. 2002. Manejo integrado de plagas y enfermedades. *In* Pumisacho, M.; Sherwood, S. El cultivo de la papa en Ecuador. Quito, INIAP/CIP. Pp. 85-169.

Pérez, W., Ñahui, M., Ellis, D., and Forbes, G. A. 2014. Wide phenotypic diversity for resistance to Phytophthora infestans found in potato landraces from Peru. Plant Disease 98:1530-1533.

Revelo J., Garcés S., Andrade J. 1997a. Identification of sources of horizontal resistance to *Phytophtora infestans* free of major genes. CIP Circular (April): 15-17.

Revelo J., Garcés S., Andrade J. 1997b. Resistance of commercial potato varieties to attack by Phytophthora infestans in Ecuador. CIP Circular (April): 20-21.

Revelo J., Andrade J., Garcés, S. 1997. El tizón tardío o lancha de la papa problema permanente de los agricultores. In Memorias del curso "Manejo integrado de las principales plagas y enfermedades del cultivo de la papa". INIAP-E.E. Santa Catalina, Quito, Ecuador. June 3 -5, 1997. Pp. 14-16.

Rogozina, E.V., Kuznetsova, M.A., Patrikeyeva, M.V., Spiglazova, S.Y., Smetanina, T.I., Semenova, N.N., Deahl, K. 2010. Late blight-resistant tuber-bearing Solanum species in field and laboratory trials. *In* Twelfth EuroBlight workshop. Arras, France, May 3-6, 2010. Pp. 239-246.

Shaner G., Finney R.E. 1977. The Effect of Nitrogen Fertilization on the Expression of Slow-Mildewing Resistance in Knox Wheat. Phytopathology 67: 1051-1056.

Sharma, B.P., Forbes, G.A., Manandhar, H.K., Shrestha, S.M., Thapa, R.B. 2013. Determination of resistance to *Phytophthora infestan son* potato plants in field, laboratory and greenhouse conditions. Journal of Agriculture Science 5 (5), 148-157.

Sliwka, J., Zimnoch-Guzowska. E. 2013. Resistance to late blight. Chapter 12. In Varshney R.K. & Tuberosa R. (eds.). Translational Genocis for Crop Breeding. Volume 1: Biotic stress. John Wiley & Sons. Pp. 221-240.

Tello, C. 2008. Identificación de aspectos epidemiológicos relacionados con la expresión de resistencia de la papa (*Solanum tuberosum*) para las poblaciones de *Phytophthora infestans* predominantes en tres localidades de la sierra ecuatoriana. Tesis de grado. Quito, Universidad Central del Ecuador. 95p.

Thurston, H.D., Heidrick, L.E., Guzman, J. 1962. Partial resistance to *Phytophthora infestans* (Mont.) De Bary within the Coleccion Central Colombiana. American Potato Journal 39, 63-69.

Vleeshouwers, V.G.A.A., Dooijeweert, W., Keizer, L.C.P., Sijpkes, L., Govers, F., Colon, L.T. 1999. A laboratory assay for Phytophthora infestans resistance in various Solanum species reflects the field situation. European Journal of Plant Pathology 105: 241–250.

Figure 1. Inoculated potato leaflets (A); Necrotic lesion measured with ImageJ software (B); Leaflet area measured with ImageJ software (C).

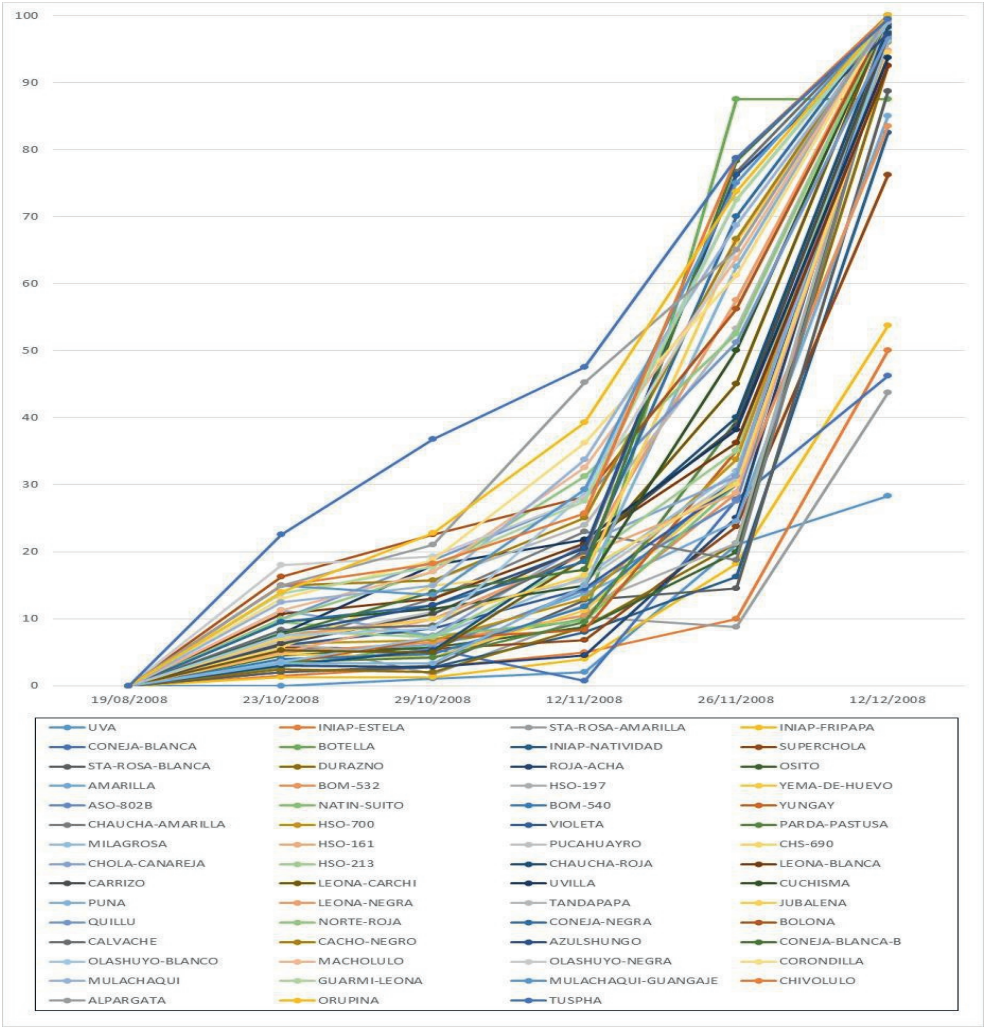


Figure 2. Severity (%) in fifty-five potato clones under natural infection conditions in Carchi, Ecuador (Sowing date: 19/08/2008).

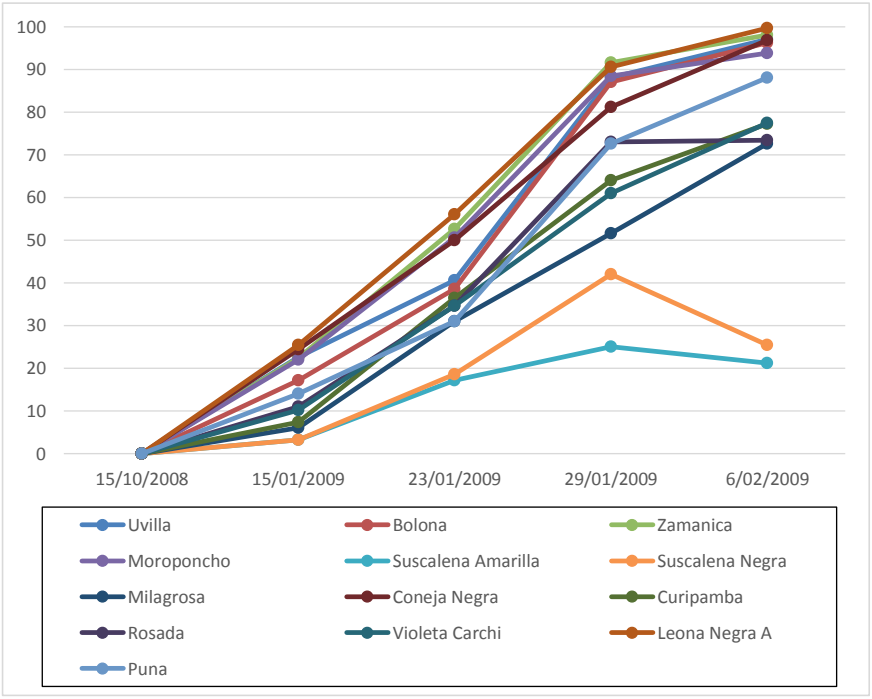


Figure 3. Severity (%) in fifteen Ecuadorian potato landraces under natural infection conditions in Carchi, Ecuador (Sowing date: 15/10/2008).

Table 1. Area under the disease progress curve (AUDPC) of fifty-two Ecuadorian potato landraces under natural infection conditions in Carchi, Ecuador.

Genotype	Type[a]	AUDPC[b]	
Santa Rosa Amarilla	lan	574.33	A
Uva	lan	579.67	A
INIAP-Estela	cv	661.33	A
INIAP-Fripapa	cv	804.00	A
Coneja-Blanca	lan	876.00	A
Botella	lan	965.00	A
INIAP-Natividad	cv	1046.33	A
Superchola	cv	1183.00	B
Santa-Rosa-Blanca	lan	1221.67	B
Amarilla[c]	lan	1248.00	B
Roja-Acha	lan	1261.67	B
Durazno	lan	1265.00	B
Osito	lan	1318.00	B
HSO-197	lan	1324.33	B
BOM-540	lan	1419.00	B
Natin-Suito[c]	lan	1480.00	B
Yema-de-Huevo	lan	1483.00	B

Yungay	lan	1495.00	B	
BOM-532	lan	1514.00	B	
Parda-Pastusa	lan	1517.33	B	
ASO-802B	lan	1534.67	B	
Puca-Huayro[c]	lan	1547.50	B	
Chaucha-Amarilla	lan	1567.33	B	
HSO-700	lan	1620.67	B	
Violeta	lan	1661.00	B	
CHS-690	lan	1675.33	B	
HSO-161	lan	1694.67	B	
Milagrosa	lan	1713.00	B	
Uvilla	lan	1751.33	B	
Cuchisma	lan	1802.00	B	
Carrizo	lan	1808.67	B	
HSO-213	lan	1810.33	B	
Chaucha-Roja	lan	1832.67	B	
Chola-Cañareja	lan	1863.00	B	
Leona-Blanca	lan	1939.67		C
Leona-Carchi	lan	1945.67		C
Puña	lan	1982.59		C
Leona-Negra	lan	2194.33		C
Tandapapa	lan	2232.50		C
Norte-Roja	lan	2276.00		C
Bolona	lan	2335.67		C
Quillo	lan	2339.67		C
Calvache	lan	2345.00		C
Jubaleña	lan	2347.33		C
Cacho-Negro	lan	2351.67		C
Coneja-Negra	lan	2441.00		C
Olashuyo-Negra	lan	2453.33		C
Coneja-Blanca-B	lan	2470.00		C
Azulshungo	lan	2566.00		C
Mulachaqui-Guangaje	lan	2578.33		C
Guarmi-Leona	lan	2581.67		C
Corondilla	lan	2587.00		C
Mulachaqui	lan	2593.33		C
Macholulo	lan	2630.00		C
Olashuyo-Blanco	lan	2631.67		C
Chivolulo	lan	2657.33		C
Alpargata	lan	2790.67		C
Orupina	lan	2830.00		C
Tuspha	lan	3048.33		C
C.V. (%)= 22.50				

[a] lan= landrace, cv= commercial variety.
[b] Means followed by the same letter are not significantly different (α=0.05) according to Scott & Knott test.
[c] Landraces from other countries: Natin Suito (CIP702464) from Colombia, Amarilla (CIP704481) from Perú, Puca Huayro (CIP 701524) from Perú.

Table 2. Area under the disease progress curve (AUDPC) of fifteen Ecuadorian potato landraces under natural infection conditions in Carchi, Ecuador.

Genotype	AUDPC[a]			
Suscaleña Amarilla	369.00	A		
Suscaleña Negra	483.67	A		
Milagrosa	891.00		B	
Rosada Carchi	943.00		B	
Curipamba	987.33		B	
Violeta Carchi	1017.00		B	
Puña	1075.67		B	
Rosada	1092.67		B	
Leona Negra A	1129.67		B	
Bolona	1238.67			C
Coneja Negra	1302.33			C
Uvilla	1302.67			C
Zamanica	1340.67			C
Moroponcho	1362.67			C
Leona Negra B	1476.00			C
C.V. (%)= 12.32				

[b] Means followed by the same letter are not significantly different (α=0.05) according to Scott & Knott test.

Table 3. Percentage of diseased area on detached leaflets in five landraces and two potato varieties inoculated with three different isolates of *Phytophthora infestans*.

Genotypes	Isolates [a, b]								
	IC 6.2			ICh 21.9			IL 4.1		
AXC-25	0.42	A		0.64	A		0.59	B	
Superchola[c]	0.42	A		0.50	A		0.49	A	
AC-37	0.47	A		0.73		B	0.62	B	
INIAP-Fripapa[c]	0.64		B	0.67	A		0.45	A	
AMFY-16	0.66		B	0.75		B	0.78		C
MG-7	0.67		B	0.84		B	0.79		C
AXC-7	0.83		B	0.84		B	0.80		C
C.V. (%)	20.23			14.45			11.36		

[a] Values of Percentage of diseased area were transformed by square root. Means in columns followed by the same letter are not significantly different (α=0.05) according to Scott & Knott test.
[b] IC6.2 isolate is from Carchi province and its race is 1, 3, 4, 7, 10; Ich21.9 isolate is from Chimborazo province and its race is 1, 4, 7, 9, 10, 11; IL4.1 9 isolate is from Loja province and its race is 1, 4, 7, 8, 9, 10, 11.
[c] Commercial varieties.

Chapter 5

Farmers' perception of Ecuadorian potato landraces

Alvaro Monteros-Altamirano[1,2,4,5], Ricardo A. Delgado[1,2,4,5], Ronald Van Den Berg[3], Richard G.F. Visser[2], Ben Vosman[2]

[1]Instituto Nacional Autónomo de Investigaciones Agropecuarias INIAP. Estación Experimental Santa Catalina. Panamericana Sur Km 1. Quito, Ecuador.
[2]Plant Breeding, Wageningen University and Research, P.O. Box 386, 6700 AJ Wageningen, The Netherlands.
[3]Biosystematics Group, Wageningen University, P.O. Box 647, 6700 AJ Wageningen, The Netherlands.
[4]The Graduate School Experimental Plant Sciences, Wageningen University, Wageningen, The Netherlands.
[5]Both authors contributed equally to this paper.

Submitted for publication

Abstract

A field experiment was carried out to assess resistance or susceptibility to late blight of 31 Ecuadorian potato landraces from a new germplasm collection obtained in Carchi, Chimborazo and Loja. The experiment was conducted in Quito at the Santa Catalina Experimental Station (EESC) of the National Institute for Agricultural Research (INIAP). This location was selected because it is under high *P. infestans* pressure. Additionally, a survey of 145 farmers growing potato landraces in these three provinces identified the main diseases affecting their potatoes. Informal conversations with these farmers both during the collections and during farmer meetings provided additional information regarding late blight and their perception of landrace resistance. The landraces under study showed different responses to late blight in the experimental field. Based on the AUDPC scores we distinguish three categories: resistant, moderately resistant and susceptible. Five landraces (and one commercial variety grown as control) showed the best field resistance. Similar to farmers growing commercial varieties also farmers currently cultivating landraces consider late blight as the main disease in their potatoes. It is interesting that farmers have managed to maintain these mostly susceptible landraces for centuries. Probably the broad crop diversity on their farms and the planting of potato landrace mixtures reduces the late blight severity effects within their potato fields. Possible strategies to improve late blight resistance in potato in Ecuador could include the identification of accessions with resistance among local landraces and/or the introduction of new sources of resistance from other origins. Alternatively, one could attempt to introduce novel R-genes in material that already contains some level of field or quantitative resistance.

Keywords: late blight, *Phytophthora infestans*, resistance

Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases of potato world-wide (Birch & Whisson, 2001). The disease is also a limiting factor in potato production in Ecuador (Crissman *et al.*., 1998). It has been observed that under extreme climatic conditions, the potato crop can be destroyed within a few days after the first symptoms are visible (Oyarzún *et al*., 2001). All the information on the importance of late blight is based on commercially grown potatoes in Ecuador. However, Ecuadorian farmers also maintain potato landraces in their fields (Monteros-Altamirano, 2011). These landraces have endured biotic and abiotic stresses for generations and are still maintained under low input conditions.

Resistance to late blight may be based on vertical resistance or horizontal resistance. Vertical resistance is based on major genes which include amongst others the so-called NBS-LRR type of resistance genes. Such resistance (R)-genes often originate from wild relatives of potato (van der Vossen, 2003; Tan *et al*., 2008; Pel *et al*.., 2009; Jacobs *et al*., 2010; Lokossou *et al*., 2010). Horizontal resistance, also known as quantitative or field resistance, is based on multiple genes that each have a relatively small effect and, in theory, render the host partially resistant to all races of the pathogen (Vanderplank, 1968; Turkensteen, 1993; Colon *et al*., 1995; Landeo *et al*., 1995). Some authors consider field resistance more stable than resistance based on R-genes (Turkensteen, 1993; Wulff *et al.*, 2007; Brown & Caligari, 2008). However, also pyramiding of R-genes has been suggested as a strategy for obtaining late blight resistance (Tan *et al*., 2010).

The Andean cultivated potatoes *S. tuberosum* ssp. *andigenum* and *S. phureja* are reported to have quantitative resistance (Simmonds & Malcomsom, 1967; van Soest *et al*., 1984; Turkensteen, 1993; Cañizares & Forbes, 1995). Van Soest *et al*., (1984) evaluated nearly 200 accessions of *S. tuberosum* ssp. *andigenum* and found intermediate to high susceptibility to late blight. Based on this they concluded that these materials have no practical value for breeding. Gabriel *et al*., (2007) found good quantitative resistance in *S. tuberosum* ssp. *andigenum* accessions when compared to accessions of *S. stenotomum*, *S. juzepczukii* and *S. ajanhuiri* from Bolivia. Also, late blight resistance was found in *S. tuberosum* ssp. *andigenum* seedling populations under long-day conditions after mass-selection (Simmons & Malcoms, 1967). Van Soest *et al*., (1984) found resistance in one accession of *S. phureja*. In evaluations of Ecuadorian landraces of *S. phureja*, mostly susceptible material was found, but also some accessions with field resistance to late blight were identified (Cañizares & Forbes, 1995; Revelo *et al*., 1997a). One of the resistant accessions of *S. phureja* (CHS-625) was crossed with a susceptible *S. tuberosum* DH line (PS-3) producing a dihaploid hybrid population that segregated for quantitative resistance (Trognitz *et al*., 2001). In this population QTLs associated with field resistance to late blight under short-day conditions were identified (Ghislain *et al*., 2001; Trognitz *et al*., 2002). Also two PR-1 genes have been isolated and proposed to play a role in horizontal late blight resistance in *S. phureja* (Evers, 2006). In conclusion, Andean potatoes show quite some variation in resistance to late blight, but unfortunately many of them are susceptible. However, field resistance does exist and accessions with this characteristic have been used in breeding programs.

In this study we evaluate selected Ecuadorian landraces from three provinces for late blight resistance under natural conditions. We connect our evaluation with the farmers' perception of their landraces in relation to late blight resistance and their understanding of potato landrace management in the field.

Materials and methods

Plant materials

We studied 31 Ecuadorian potato landraces collected in the provinces of Carchi, Chimborazo and Loja, which are areas of high potato diversity (Monteros-Altamirano, 2011). Three of these landraces are classified as *S. tuberosum* diploid Andigenum Group (Spooner *et al*., 2007), formerly *S. phureja* (Hawkes, 1990); one landrace as *S. tuberosum* triploid Andigenum Group (Spooner *et al*., 2007), formerly *S. chaucha* (Hawkes, 1990); and 27 landraces as *S. tuberosum* tetraploid Andigenum Group (Spooner *et al*., 2007), formerly S. *tuberosum* ssp. *andigenum* (Hawkes, 1990). The ploidy levels of all the materials were confirmed by flow cytometry as described in Monteros-Altamirano (2011). The 31 landraces were selected from a set of 152 native potatoes collected recently in the provinces of Carchi, Chimborazo and Loja, which had been genotyped previously with 8 SSRs in Monteros-Altamirano (2011). Figure 1 shows the relationship among the materials selected for this study. Additionally two improved tetraploid commercial varieties were included in the analysis as control: 'Superchola' and 'I-Fripapa'.

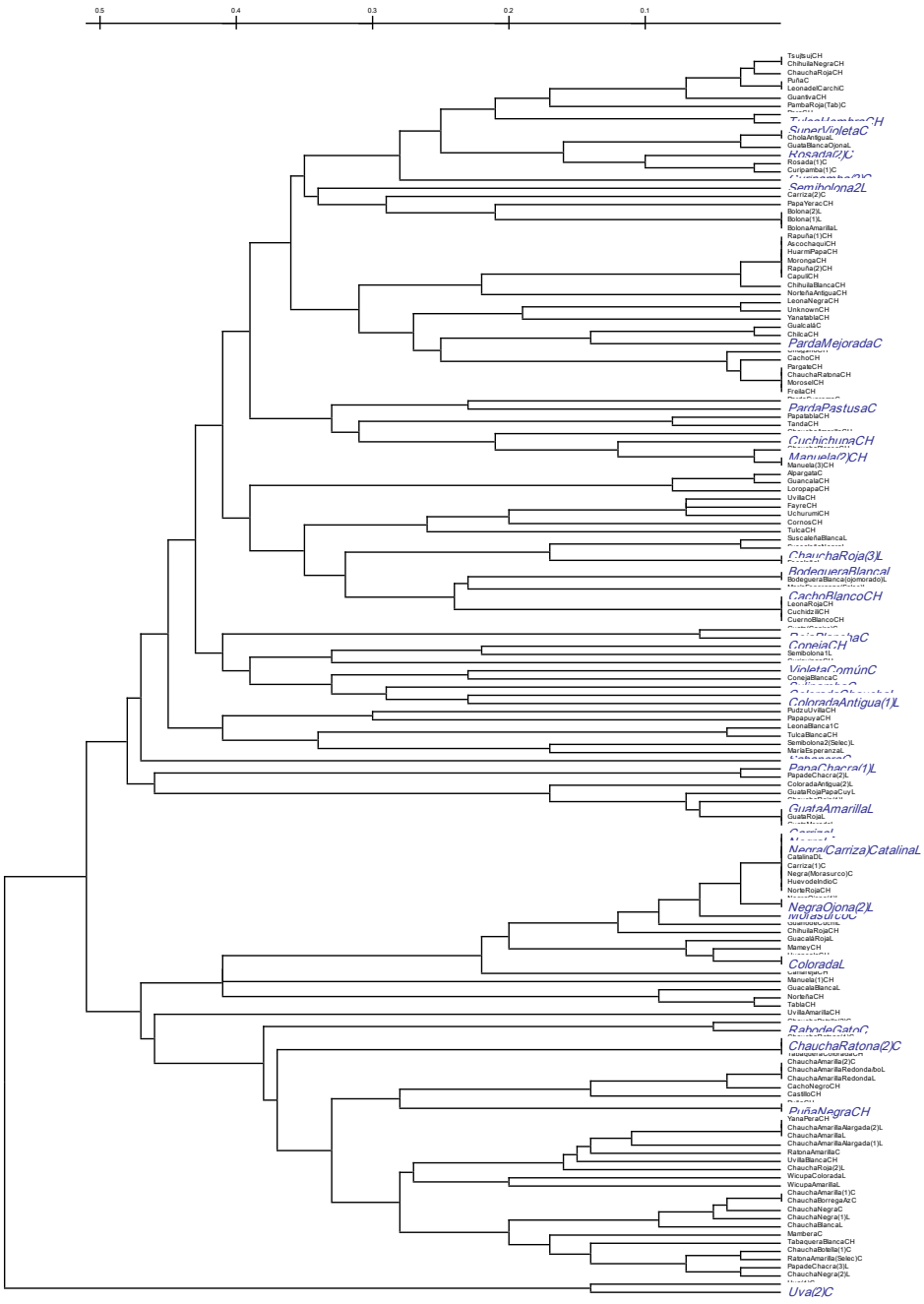


Figure 1. UPGMA tree showing the genetic relationship of the 31 landraces selected for the study (in blue). The tree was constructed using the Nei and Li coefficient (Nei and Li, 1979) based on 8 SSRs (Monteros-Altamirano, 2011).

Farmers from Carchi, which is at the border with Colombia, provided 'Parda mejorada' and 'Parda pastusa' as landraces. However, there are also Colombian commercial varieties under these names. According to Ñustez (2010) the Colombian 'Parda pastusa', was produced by a cross ['Quincha' (*S. tuberosum* ssp. *andigenum*) x 'Tocana colorada' (*S. tuberosum* ssp. *andigenum*)]. The material of 'Parda pastusa' used in our study was triploid and we consider this material as a landrace. We could not get additional information on 'Parda mejorada'. 'Uva' was collected as a landrace but turned out to be genetically distant from all other potato landraces. It apparently is a spontaneous hybrid between *S. tuberosum* ssp. *andigenum* x *S. chilotanum* (Ghislain *et al*., 2009).

Farmers´ information

145 surveys were conducted with farmers growing potato landraces in the provinces of Carchi, Chimborazo and Loja (Monteros-Altamirano, 2011). The survey included a question regarding the main diseases affecting the potato landraces (Appendix 1). Farmers provided common names of the diseases affecting their landraces. This information was compared to Oyarzún *et al*. , (2002) who described potato diseases present in Ecuador. Also, informal conversations with farmers both during the collections and during farmer meetings provided information regarding late blight and their perception of landrace resistance.

Field experiment

A field experiment was carried out to assess resistance or susceptibility of Ecuadorian potato landraces to late blight. The experiment was conducted in Quito at the Santa Catalina Experimental Station (EESC) of the National Institute for Agricultural Research (INIAP) located at 3050 m.a.s.l, Longitude: 78º33'15" and Latitude: 00º22'4" S. The average annual temperature is 13ºC, the annual precipitation: 1432.1 mm, and the relative humidity (annual average) 72.5 % (data from Izobamba Meteorological Station, in EESC). This location was selected because it is under high *P. infestans* pressure. In the past, 36 complex races of *P. infestans* were identified at this location (Tello, 2008).

A complete random block design with four repetitions was used. The landraces were planted in single row plots of ten plants per repetition, with a plant spacing of 0.25 m and a row spacing of 1.0 m. One application of contact fungicide (Mancozeb) was done after 30 days of emergence to protect the plants from complete devastation by late blight. This protocol is common practice at Santa Catalina station due to the high disease pressure. It is also recommended by the International Potato Center (2006).

The plant materials were evaluated under natural infection pressure. The severity of the foliage damage caused by late blight (as a percentage of leaf surface) was assessed every 7 days for 4 weeks. The evaluation started when the first symptoms were observed (62 days after emergence). The late blight assessments were used to calculate the Area Under the Disease Progress Curve (AUDPC) following Shaner & Finney (1977):

$$AUDPC = \sum^{n} [(Y_{i+n1} + Y_i)/2] [X_{i+1} - X_i]$$

$$\sum_{i=1}$$

in which Y_i = late blight severity (per plot) at the i*th* observation, X_i = time (days) at the i*th* observation, and n = total number of observations.

Data analysis

We used SAS (release 9.1, SAS Institute, Inc., Cary, NC) to perform an ANOVA analysis. A LSD Fisher test on the AUDPC data was performed in Infostat® (Di Rienzo *et al*., 2008) to determine the statistical significance of the differences among the landraces.

The cultivars were classified by their susceptibility to late blight based on their average AUDPC values according the following scale: highly resistant (< 250), resistant (251-550), moderately resistant (551-650), susceptible (651-1200) and highly susceptible (>1200) (Gopal & Singh, 2003).

Results

Diseases affecting Ecuadorian potato landraces

A total of 145 farmers provided information about the main diseases affecting their potato landraces (47 from Carchi, 49 in Chimborazo and 49 in Loja). Farmers mentioned nine diseases affecting their potatoes. The number of times a respondent mentioned a disease is shown in Figure 2. Late blight caused by *Phytophthora infestans*, in Ecuador known as 'Lancha', was most frequently mentioned in the three areas. The second important disease in Carchi was 'Lanosa' *(Rosellinia* sp.) and in Chimborazo and Loja: 'Pudrición de la raíz', which is root wilt (in this case the pathogen is unknown). The farmers from Loja mentioned more local common names of diseases than in the other areas, but the associated pathogens are unknown. These diseases were grouped under the category "Other".

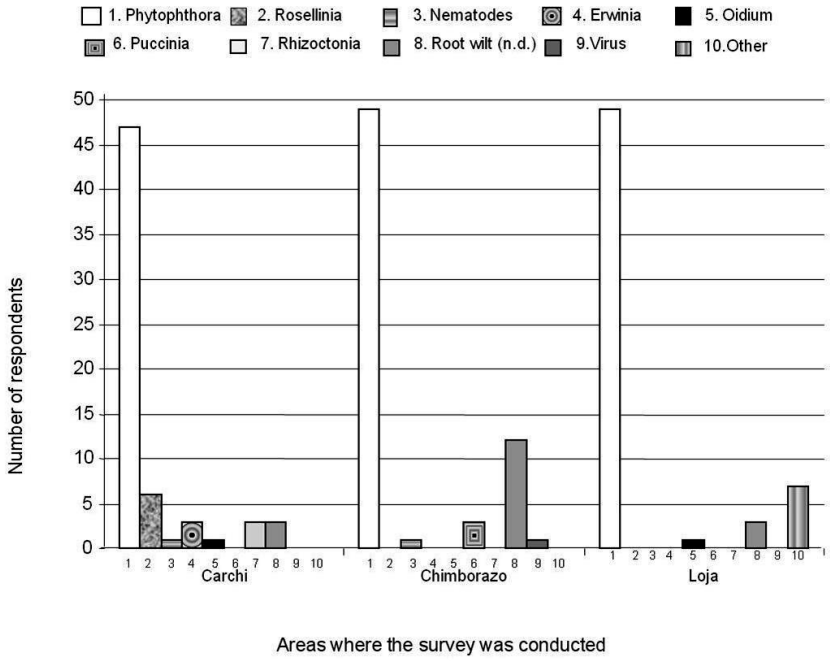


Figure 2. Number of respondents that mentioned a disease affecting potato landraces in three provinces of Ecuador (n=145). All the diseases mentioned by the farmers are included (sometimes more than one per farmer).

Response to late blight of the selected Ecuadorian landraces

The Analysis of Variance for the model AUDPC = Blocks + landraces, was highly significant (F= 4.16; P= 0.0001). The variation among blocks was not significant (F= 0.33; P= 0.8285) and the variation among landraces highly significant (F= 4.52; P= 0.0001).

The LSD test showed significant differences among the landraces according to their field response to late blight (Table 1).

Table 1. Late blight resistance of the Ecuadorian potato landraces and the two varieties. Common names of the landraces, origin, collection code, AUDPC values, LSD and ploidy levels are shown.

Landrace	Province	Code	Ploidy	AUDPC (average)	LSD*	Late blight reaction
Uva	Carchi	JS-2	4x	331	A	R
Chaucha roja	Loja	MPG-028	4x	374	AB	R
Guata amarilla	Loja	MG-007	4x	427	ABC	R
Coneja	Chimborazo	FM RA FH 002	4x	434	ABC	R
Chaucha ratona	Carchi	AXC-028	2x	505	ABC	R
Fripapa (Var.)			4x	507	ABC	R
Superchola (Var.)			4x	557	ABCD	MR
Negra, Carriza or Catalina	Loja	MOPG-002	4x	589	BCD	MR
Super violeta	Carchi	AXC-004	4x	597	BCD	MR
Violeta común	Carchi	AXC-025	4x	599	BCD	MR
Curipamba	Carchi	AXC-016	4x	628	BCDE	MR
Colorada chaucha	Loja	MOPG-004	4x	663	BCDE	S
Parda Pastusa	Carchi	AC-042	3x	692	BCDE	S
Morasurco	Carchi	AXC-009	4x	696	BCDE	S
Puña negra	Chimborazo	FM FH RA 002	4x	711	BCDEF	S
Negra ojona	Loja	MG-011	4x	714	CDEF	S
Parda mejorada	Carchi	AXC-020	4x	716	CDEF	S
Bodeguera blanca	Loja	MOPG-009	4x	723	CDEF	S
Semibolona	Loja	MG-014A	4x	759	CDEF	S
Negra	Loja	MPG-033	4x	761	CDEF	S
Colorada	Loja	MOPG-003	4x	768	CDEF	S
Carriza	Loja	MPG-020	4x	778	CDEF	S
Tulca hembra	Chimborazo	XCFM-19	4x	781	CDEFG	S
Sulipamba	Carchi	AXC-003	4x	788	DEFG	S
Colorada antigua	Loja	MPG-042	4x	842	DEFG	S
Roja plancha	Carchi	AXC-030	4x	891	DEFG	S
Papa chacra	Loja	MPG-021	2x	914	DEFG	S
Rabo de gato	Carchi	AC-040	2x	917	DEFG	S
Manuela	Chimborazo	AMFY-1	4x	917	EFG	S
Rosada	Carchi	AXC-029	4x	919	EFG	S
Cuchi chupa	Chimborazo	FMFYRA IV 005	4x	1013	FG	S
Cacho blanco	Chimborazo	FM RA FH 002	4x	1069	FG	S
Sabanera	Carchi	AC-034	4x	1125	G	S

* Different letters indicate significant difference at $\alpha = 0.05$.
** HR= Highly Resistant (AUDPC<250); R= Resistant (AUDPC 251-550); MR= Moderately Resistant (AUDPC 551-650); S= Susceptible (AUDPC 651-1200); Highly Susceptible (AUDPC >1200).

A total of five landraces and one variety were ranked as resistant. Three of these landraces are from the *S. tuberosum* tetraploid Andigenum Group: 'Uva', 'Guata amarilla', 'Coneja' and 'Chaucha roja' and one from the *S. tuberosum* diploid Group: 'Chaucha ratona'. The landrace 'Chaucha roja' is an early-sprouting potato, but tetraploid while 'Chaucha ratona' is a diploid early-sprouting landrace. The landrace 'Uva' performed the best since it had the lowest AUDPC value (331) among all the landraces evaluated. Four landraces and one variety (Superchola) were moderately susceptible. These landraces include: 'Negra-Carrizo-Catalina', 'Super violeta', 'Violeta común', 'Curipamba'. Twenty two of the landraces were susceptible

to late blight. There was no obvious link between the level of susceptibility and the origin of the different landraces.

The 145 farmers were growing some of the landraces used in our study and knew /acknowledged that indeed these landraces were susceptible to late blight. No reports were made of very conflicting results although some of the landraces used in more than one province or region were less/more susceptible in one versus the other region.

Discussion

Ecuadorian landraces and late blight performance

The landraces under study showed different responses to late blight in the experimental field. Most of them turned out to be only moderately resistant to susceptible (Table 1), which is in line with reports on landraces from other parts of the Andes (Van Soest *et al*., 1984; Birhman & Kang, 1993; Turkensteen, 1993). Five landraces (one diploid and four tetraploid) showed the best field resistance. The performance of these landraces was similar to the tetraploid variety 'I-Fripapa', which is a leading variety in Ecuador and reported as resistant (Oyarzún *et al*., 2001 a; Perez & Forbes, 2007) or moderately resistant (Cáceres *et al*., 2008). The variety 'Superchola' is believed to be susceptible, but was not significantly different from the most resistant landraces in our field experiment. The landrace 'Uva' performed best and is believed to have *S. tuberosum* ssp *andigenum* in its pedigree which may have donated its resistance (Turkensteen, 1993).

Late blight perception by farmers

Similar to farmers growing commercial varieties (Ortiz *et al*., 1999) also farmers currently cultivating landraces consider late blight as the main disease in their potatoes (Figure 2). Other diseases were mentioned but these are less important. Farmers are aware of differences in late blight response among their landraces. They know that certain landraces are more resistant or susceptible than others. For example, 'Sulipamba' is considered susceptible by the farmers, which was confirmed in our field experiment (Table 1). Similarly, 'Uva' was considered resistant by the farmer who provided the landrace.

Changes in the response of landraces to late blight have been noticed by farmers. In Carchi farmers mentioned that 'Violeta', 'Curipamba' and 'Morasurco' were the more resistant landraces in the past. In our field trial these landraces were only moderately resistant or even susceptible. These changes might be related to the appearance of more virulent races of *P. infestans*. Forbes *et al*., (1997) reported a shift in the *P. infestans* populations. The original clonal lineage US-1 was replaced by EC-1, which is a more complex race than the previous one.

Management practices of farmers

It is interesting that farmers have managed to maintain these mostly susceptible landraces for centuries. Apparently, there are other characteristics that promote the continued use of the

landraces, despite the fact that most of them are susceptible to late blight. Ortiz *et al*. (1999) already mentioned that farmers preferred particular cultivars for other reasons than late blight resistance. For example in our study the landrace Sulipamba was determined as susceptible, but local farmers appreciated its taste.

There are also management practices that decrease the impact of late blight on the potato crop. Farmers growing potato landraces do not only keep potatoes, but a much broader crop diversity on their farms (Monteros-Altamirano, 2011). This crop diversity may provide protection to diseases by inter-cropping and crop rotation (Thurston, 1990; Garret *et al*., 2001). An example is the susceptible landrace 'Papa de chacra', which is grown within corn fields "as weedy potato" with no pesticide application. Another common practice among the farmers is planting potato landraces in mixtures. This can reduce potato late blight severity as observed by Andrivon *et al.*. (2003) and Pilet *et al*. (2006). We observed different landraces of potatoes and also different ploidy levels intermixed in farmer fields (Monteros-Altamirano, 2011).

Potato landraces were managed organically in the past. The appearance of new commercial cultivars e.g. 'Superchola' and 'I-Fripapa' has brought new management practices to the commercial potatoes. A large range of fungicides and excessive use of them has been documented in commercial potatoes in Ecuador (Crissman 1994, 1998; Ortiz *et al*., 1999; Ortiz *et al*., 2001). Pesticide application on the commercial varieties is now common practice and farmers are also increasing their use of potato landraces. Currently 64% of the farmers in Carchi, 58% in Chimborazo and 60% in Loja are managing landraces similarly to commercial varieties (Monteros-Altamirano, 2011).

Finally, farmers growing landraces are aware of ways to escape late blight; e.g. farmers in Loja skip the heavy rainy season to avoid losses due to late blight attack (Monteros-Altamirano, 2011).

Perspectives for late blight resistance breeding

From this study (Table 1) and previous reports, it is clear that there is variation in the level of resistance to late blight in Ecuadorian landraces (Cañizares and Forbes, 1995; Revelo *et al.et al.*., 1997a). Possible strategies to improve late blight resistance in potato in Ecuador could include the identification of accessions with resistance among local landraces and/or the introduction of new sources of resistance from other origins. A screening of the available potato germplasm could be carried out. However, considering our results on a selection of landraces that represents the available diversity quite well (Figure 1), this might not lead to much improvement as most landraces turned out to be susceptible (Table 1).

Previous experiences with the release of varieties carrying single R-genes in Ecuador showed that the resistance was quickly overcome by the *P. infestans* population (Revelo *et al*., 1997b; Oyarzun *et al*., 2001). This probably is due to the high variability of the *P. infestans* populations present in the Ecuadorian highlands (Forbes *et al*., 1997; Tello, 2008). As an alternative, the pyramiding of novel R-genes obtained from different sources has been proposed to improve late blight resistance and its durability (Tan *et al*., 2010; Verzaux, 2010). However, we have to keep in mind that several of the R-genes have already been defeated. Therefore, a careful selection has to be made based on the frequency of the different races and composition of the *P. infestans* population. It is encouraging that recent research identified several novel R-genes in wild tuber bearing *Solanum* species that will be useful (Wang *et al*.,

2008; Jacobs *et al*., 2010; Pel *et al*., 2009, Jo *et al*, 2011; Rietman *et al.*, 2012; Jo *et al.*, 2015). In addition, it might be a viable strategy to introduce these novel R-genes in material that already contains some level of quantitative resistance, as suggested by Stewart *et al*. (2003).

Acknowledgments

To the Netherlands Organization for International Cooperation in Higher Education (NUFFIC), the Secretaria Nacional de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT), and the Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP) for the financial support.

Literature cited

Andrivon D., Lucas J.M., Ellissèche D. 2003. Development of natural late blight epidemics in pure and mixed plots of potato cultivars with different levels of partial resistance. Plant Pathology 52: 586-594.

Birch P.R.J., Whisson S.C. 2001. Phytophthora infestans enters the genomics era. Molecular Plant Pathology 2 (5): 257-263.

Birhman R.K., Kang G.S. 1993. Analysis of variation and interrelationships in potato germplasm. Euphytica 68: 17-26.

Brown J., Caligari P. 2008. An introduction to plant breeding. Blackwell Publishing: 209.

CIP. 2006. International Potato Center. Procedures for standard evaluation trials of advanced potato clones. An International Cooperators' Guide.

Cáceres P., Pumisacho M, Forbes G., Andrade-Piedra J. 2008. Learning to control potato late blight - A facilitator's guide. International Potato Center (CIP), Instituto Nacional 115 Autónomo de Investigaciones Agropecuarias (INIAP), Secretaría Nacional de Ciencia y Tecnología (SENACYT): 142.

Cañizares, C.A. , Forbes, G.A. 1995. Foliage resistance to *Phytophthora infestans* (Mont.) de Bary in the Ecuadorian national collection of *Solanum phureja* ssp.*phureja* Juz. & Buk Potato Research 38: 3-10.

Colon L.T., Turkensteen L.J., Prummel W., Budding D.J., Hoogendoorn J. 1995. Durable resistance to late blight (Phytophthora infestans) in old potato cultivars. European Journal of Plant Pathology 101: 387-397.

Crissman C., Espinosa P., Ducrot C., Cole D., Carpio F. 1998. The case study site: Physical, Health, and Potato Farming Systems in Carchi province. In: Crissman C., Antle J., and Capalbo S. (Eds.). Economic, environmental and health tradeoffs in agriculture: Pesticides and the sustainability of Andean potato production. Kluwer Academic publisher in cooperation with the International Potato Center. Massachusetts, USA: 85-117.

Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. 2008. InfoStat, versión 2008, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Evers D., Schweitzer C., Nicot N., Gigliotti S., Herrera M.R., Hausman J.F., Hoffmann L. Trognitz B., Dommes J., Ghislain M. 2006. Two PR-1 loci detected in the native cultivated potato *Solanum phureja* appear differentially expressed upon challenge by late blight. Physiological and Molecular Plant Pathology 67: 155-163.

Forbes G.A., Escobar X.C., Ayala C.C., Revelo J., Ordoñez M.E., Fry B.A., Doucett K., Fry W.E. 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. Phytopathology 87: 375-380.

Gabriel J., Coca A., Plata G., Parlevliet J.E. 2007. Characterization of the resistance to *Phytophthora infestans* in local potato cultivars in Bolivia. Euphytica 153: 321-328.

Garrett K.A., Nelson R.J., Mundt C.C., Chacón G., Jaramillo R.E., Forbes G.A. 2001. The effects of host diversity and other management components on epidemics of potato late blight in the humid highland tropics. Phytopathology 91: 993-1000.

Ghislain M., Núñez J., Herrera M.R., Spooner D.M. 2009. The single Andigenum origin of Neo-Tuberosum potato materials is not supported by microsatellite and plastid marker analyses. Theor Appl Genet 118: 963-969

Ghislain M., Trognitz· B.,·Herrera Ma. del R., Solis J., Casallo G., Vásquez C., Hurtado O., Castillo R., Portal L., Orrillo M. 2001. Genetic loci associated with field resistance to late blight in offspring of *Solanum phureja* and *S. tuberosum* grown under short-day conditions. Theor Appl Genet 103: 433-442.

Gopal, J., Singh, B.P. 2003. Screening potatoes for resistance to late blight (*Phytophthora infestans*) under field conditions. *Potato Res* **46**: 47-56.

Hawkes J.G. 1990. The potato-evolution, biodiversity and genetic resources. Smithsonian Institution Press, Washington DC: 259.

Jacobs M.M.J., Vosman B., Vleeshouwers V.G.A.A., Visser R.G.F., Henken B., Van den Berg R.G. 2010. A novel approach to locate Phytophthora infestans resistance genes on the potato genetic map. Theor. Appl. Genet. 120: 785-796.

Jo K-R, J Vossen, E Jacobsen, MA Jongsma, RGF Visser, T-Y Kim & S-H Kang (2011). Mapping of the S. demissum late blight resistance gene R8 to a new locus on chromosome IX. Theor. Applied Genet. 123:1331–1340.

Jo K-R, RGF Visser, E Jacobsen & J Vossen (2015). The *R9* potato late blight resistance is conferred by quantitative resistance and a qualitative locus residing in a cluster of Tm-2 homologs on chromosome IX. Theor Appl Genet 128:931–941.

Landeo J.A., Gastelo M., Pinedo H., Flores F. 1995. Breeding for horizontal resistance to late blight in potato free of R-genes. In: Dowley L.J., Bannon E., Cooke L.R., Keane T. O'Sullivan E. (Eds.). Phytophthora infestans 150. Boole Press, Dublin, Eire: 268- 274.

Lokossou A.A., Rietman H., Wang M., Krenek P., Van der Schoot H., Henken B., Hoekstra R., Vleeshouwers V.G.A.A., Van der Vossen E.A.G, Visser R.G.F, Jacobsen E., Vosman B. 2010. Diversity, distribution and evolution of *Solanum bulbocastanum* Late Blight resistance genes. MPMI 23: 1206-1216.

Monteros-Altamirano A, 2011. *Potato landraces: description and dynamics in three areas of Ecuador.* Wageningen, The Netherlands: Wageningen University, PhD Thesis.

Ñustez C. 2010. Variedades Colombianas de papa. Universidad Nacional de Colombia. Grupo de Investigación en Papa. http://papaunc.com/catalogo.shtml. (Accessed: 25/12/2010).

Ortiz O., Winters P., Fano H., Thiele G., Guamán S., Torres R., Barrera V., Unda J., Hakiza J. 1999. Understanding Farmer's response to late blight: Evidence from Peru, Bolivia, Ecuador and Uganda. In: CIP Program Report, 1997-1998. International Potato Center, Lima-Peru: 101-110.

Ortiz O., Thiele G., Forbes G. 2001. Farmer's knowledge and practices regarding fungicide use for late blight control in the Andes. In: Fernández-Northcote E.N. (Ed.). Proceedings of the International Workshop Complementing resistance to late blight Phytophthora infestans in the Andes. February 13-16, 2001. Cochabamba-Bolivia: 45- 56.

Oyarzún P.J., Taipe J.A., Forbes G.A. 2001. *Phytophthora infestans* characteristics and activity in Ecuador. Country profile. In: Fernández-Northcote E.N. (Ed.). Proceedings of the International Workshop Complementing resistance to late blight Phytophthora infestans in the Andes. February 13-16, 2001, Cochabamba-Bolivia: 17-28.

Oyarzún P., Gallegos P., Asaquibay, C. Forbes G., Ochoa J., Paucar B., Prado M., Revelo J., Sherwood S., Yumisaca F. 2002. Manejo integrado de plagas y enfermedades. In: Pumishacho M., and Sherwood S. (Eds.). El cultivo de la papa en el Ecuador. INIAPCIP: 85-131.

Pel M.A., Foster S.J., Park T.H., Rietman H., Van Arkel G., Jones J.D.G., Van Eck H.J., Jacobsen E., Visser R.G.F., Van der Vossen E.A.G. 2009. Mapping and cloning of Late Blight resistance genes from *Solanum venturii* using an interspecific candidate gene approach. MPMI 22 (5): 601-615.

Perez W., Forbes G. 2007. Qué es la resistencia genética? Manejo integrado del Tizón Tardío. Hoja divulgativa 3. Centro Internacional del Papa: 4.

Pilet F., Chacón G., Forbes G.A., Andrivon D. 2006. Protection of Susceptible Potato Cultivars Against Late Blight in Mixtures Increases with Decreasing Disease Pressure. Phytopathology 96 (7): 777-783.

Revelo J., Garcés S., Andrade J. 1997a. Identification of sources of horizontal resistance to *Phytophtora infestans* free of major genes. CIP Circular (April): 15-17.

Revelo J., Garcés S., and Andrade J. 1997b. Resistance of commercial potato varieties to attack by *Phytophtora infestans* in Ecuador. CIP Circular (April): 20-21.

Rietman H, G Bijsterbosch, LM Cano, H-R Lee, JH Vossen, E Jacobsen, RGF Visser, S Kamoun & VGAA Vleeshouwers (2012). Qualitative and quantitative late blight resistance in the potato cultivar Sarpo Mira is determined by the perception of five distinct RXLR effectors. MPMI 25: 910–919.

Shaner G., Finney R.E. 1977. The Effect of Nitrogen Fertilization on the Expression of Slow-Mildewing Resistance in Knox Wheat. Phytopathology 67: 1051-1056.

Spooner D., Nuñez J., Trujillo G., Herrera R., Guzmán F., Ghislain M. 2007. Extensive simple sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. PNAS 104 (49): 19398-19403.

Stewart H.E., Bradshaw J.E., Pande B. 2003. The effect of the presence of R-genes for resistance to late blight (*Phytophthora infestans*) of potato (*Solanum tuberosum*) on the underlying level of field resistance. Plant Pathology 52: 193-198.

Tan M.Y.A., Hutten R.C.B., Celis-Gamboa B.C., Park T.H., Niks R.E., Visser R.G.F., Van Eck H.J. 2008. The Rpi-mcd1 Locus from *Solanum microdontum* involved in resistance to *Phytophthora infestans*, causing a delay in infection, maps on potato chromosome 4 in a cluster of NBS-LRR genes. Mol. Plant-Microbe Interact 21: 909-918.

Tan M.Y.A., Hutten R.C.B., Visser R.G.F., Van Eck H.J. 2010. The effect of pyramiding *Phytophthora infestans* resistance genes RPi-mcd1 and RPi-ber in potato. Theor Appl Genet 121: 117-125.

Tello C. 2008. Identificación de aspectos epidemiológicos relacionados con la expresión de resistencia de la papa (*Solanum tuberosum*) para las poblaciones de *Phytophthora infestans* predominantes en tres localidades de la sierra ecuatoriana. Tesis Ing. Agr. Universidad Central del Ecuador: 95.

Trognitz B.R., Orrillo M., Portal L., Román C., Ramón P., Perez S., Chacón G. 2001. Evaluation and analysis of reduction of late blight disease in a diploid potato population. Plant Pathology 50: 281-291.

Trognitz F., Manosalva P., Gysin R., Niño-Liu D., Simon R., Herrera M.R., Trognitz B., Ghislain M., Nelson R. 2002. Plant Defense Genes Associated with Quantitative Resistance to Potato Late Blight in *Solanum phureja* × Dihaploid *S. tuberosum* Hybrids. MPMI 15 (6): 587-597.

Thurston D. 1990. Plant disease Management practices of traditional farmers. Plant Disease 74 (2): 96-101.

Turkensteen L.J. 1993. Durable resistance of potatoes against Phytophthora infestans. In: Jacobs T. and Parlevliet J.E. (Eds.). Durability of disease resistance. Kluwer Academic Publishers. Dordrecht. The Netherlands: 115-124

Van der Vossen E., Sikkema A., Hekkert B.T.L., Gros J., Stevens P., Muskens M., Wouters D., Pereira A., Stiekema W., Allefs S. 2003. An ancient R gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. Plant J. 36: 867-882.

Vanderplank J.E. 1968. Disease Resistance in Plants. New York: Academic Press.

Van Soest L.J.M., Schöber B., Tazelaar M.F. 1984. Resistance to *Phytophthora infestans* in tuber-bearing species of Solanum and its geographical distribution. Potato Research 27: 393-411.

Verzaux E. 2010. Resistance and susceptibility to late blight in *Solanum*: gene mapping, cloning and stacking. PhD Thesis, Wageningen University, Wageningen, NL: 144

Wang M., Allefs S., Van den Berg R.G, Vleeshouwers V., Van der Vossen E., Vosman B. 2008. Allele mining in Solanum: conserved homologues of Rpi-blb1 are identified in *Solanum stoloniferum*. Theor. Appl. Genet. 116: 933–943.

Wulff E.G., Perez W., Nelson R.J., Bonierbale M., Landeo J.A., Forbes G.A. 2007. Identification of stable resistance to *Phytophthora infestans* in potato genotypes evaluated in field experiments in Peru. Expl Agric (43): 353-363.

Appendix 1. Questionnaire

1. Farmer name:
2. Age:
3. Race: 1= mestizo, 2=indigenous
4. Education level: 0=none, 1=primary, 2=secondary, 3=university
5. Do you have another job besides agriculture? 0=only agriculture, 1= grow minor animals, 2= cattle, 3=housekeeping, 4=paid labor, 5=other
6. Which one is more important? 1=agriculture, 2=equal, 3= other activities
7. Province: 1= Carchi, 2= Chimborazo= 3 Loja
8. Canton:
9. Parish:
10. Locality:
11. Community:
12. Size of the farm (ha):
13. Observations:
14. Date:
15. How many members of the family are men?
16. How many members of the family are women?
17. How many members of the family are working directly in agriculture?
18. How many members of the family have migrated to look for a job different than agriculture?
19. Who prepares the land? 1= men, 2= women, 3= men + women, 4= hired labor, 5= tractor, 6=. *partidario**, 7= men + hired labor, 8= men + women + hired labor
20. Who takes care of the crop daily? 1= men, 2= women, 3= men + women, 4= hired labor, 5= tractor, 6=. *partidario*, 7= men + hired labor, 8= men + women + hired labor
21. Who applies fungicides? 0= not applied, 1= men, 2= women, 3= men + women, 4= hired labor, 5= tractor, 6=. *partidario*, 7= men + hired labor, 8= men + women + hired labor
22. Who harvests? 1= men, 2= women, 3= men + women, 4= hired labor, 5= tractor, 6=. *partidario*, 7= men + hired labor, 8= men + women + hired labor, 9= all family
23. Who sells? 0= do not sell, self consumption, 1= men, 2= women, 3= men + women, 4= hired labor, 5= tractor, 6=. *partidario*, 7= men + hired labor, 8= men + women + hired labor
24. Invisible work for women:
25. Crops in the farm:
26. Is there any difference among the management of commercial potatoes and native ones? 1= Yes, 2= No
27. Potato diseases:
28. Potato plagues:
29. Grow the landraces mixed or separated? 1= mixed, 2= separated
30. If you lose your landrace, do you try to recover it? 1= Yes, 2= No
31. If you sell these landraces, where do you do it? 1= local market, 2= other
32. Do you exchange seeds with the neighbors? 1= Yes, 2= No
33. Do you know anybody that still has these local potato landraces? 1= Yes, 2= No
34. If you choose one of the lost landraces, which would you choose to get it back?
35. Do you believe if you grow potatoes together, they hybridize? 1= Yes, 2= No
36. Do you collect berries from the field and plant them?
37. Have you seen wild potatoes close to your farm field? 1= Yes, 2= No
38. Do you believe that wild species can hybridize with the cultivated ones? 1= Yes, 2= No
39. Common names of the wild potatoes:
40. Use of potato wild species:

* *partidario*, is a local name referred to a farmer that grows the crop in another farmers' land and they share the profits according to the negotiation process.

Source: Monteros-Altamirano, 2011.

Chapter 6

Construction of a linkage map using DArT markers and search for QTLs for late blight resistance in a progeny of a cross between two Ecuadorian potato landraces

Ricardo A. Delgado[1,2,3], Richard G.F. Visser[2]

[1] Instituto Nacional Autónomo de Investigaciones Agropecuárias (INIAP), Estación Experimental Santa Catalina, Panamericana Sur km 1, Quito, Ecuador.
[2] Plant Breeding, Wageningen University and Research, P.O. Box 16, 6700 AA, Wageningen, The Netherlands.
[3] Graduate school Experimental Plant Sciences, Wageningen University

Abstract

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is the most devastating potato disease in Ecuador. Main breeding goals are centered on obtaining varieties with good levels of resistance. One potential resource available for this purpose are potato landraces. A segregating population was created with two *Solanum phureja* accessions. Field experiments were conducted and late blight severity was recorded and AUPDC was calculated for each of the individuals from the crossing population. Diversity Arrays Technology (DArT) was used to genotype the individuals of the population. A male and female linkage map was constructed. The Kruskal-Wallis non-parametric test identified nineteen markers potentially associated to field resistance against blight. Interval mapping and Permutation tests were performed in order to confirm the presence of Quantitative Trait Loci (QTLs) in the population under study. No QTLs were identified by Interval mapping. Although no clear QTL with large effect could be observed some association was observed with the Kruskal-Wallis test. A low heritability was observed so most of the variation should be attributed to environmental effects. Further studies with more evaluation sites and better genome coverage should lead to confirmation of the absence or the presence of QTLs for resistance in this cross and to the answer whether the level of resistance conveyed in these type of crosses is sufficient to be of practical use.

Introduction

Late blight (LB), caused by the oomycete *Phytophthora infestans* (Mont.) de Bary is the most devastating potato disease not only in Ecuador (Oyarzún *et al*, 2002), but worldwide (Thurston & Schultz, 1981). Yield losses due to this disease can be enormous depending on the weather conditions and host resistance (Morales *et al*, 1995). The deployment of improved varieties is one of the management strategies used to reduce damage by the pathogen, Breeding efforts to obtain varieties with resistance are continuously being carried out in the country, but it takes several years until a new variety is ready for release (Cuesta, 2011).

Sources of resistance to LB used in potato breeding in Ecuador in the past included *S. demissum* and *S. pausissectum* species. Their resistance was based on major genes (R genes) that soon were overcome by the population of *P. infestans* (Revelo *et al*., 1997; Oyarzún *et al*., 2001) present in Ecuador, which consists of highly complex races (Forbes *et al.*, 1997, Tello, 2008, Chapter 3; Delgado *et al.*, 2013).

An alternative may be the use of Ecuadorian landraces. It is estimated that there are over 400 potato landraces in Ecuador (Cuesta *et al.*, 2005). These landraces are the result of selection and conservation carried out by small farmers in the highlands for many generations (Cuesta *et al.*, 2005). These potatoes have been cultivated under conditions different from the commercial varieties, mainly on a small scale with low use of pesticides (Cuesta *et al.*, 2005, Monteros & Reinoso, 2010). These landraces include species such as *Solanum phureja, S. chaucha* and *S. andigena* (Monteros-Altamirano, 2011). Early reports have identified *S. phureja* accessions as a source of quantitative resistance to late blight in Ecuador (Cañizares & Forbes, 1995, Revelo *et al.*, 1997, Garofalo, 2005), in Colombia (Escallon *et al.*, 2005; Mosquera, 2007) and more recently in Bolivia (Gabriel *et al.*, 2013). Quantitative trait loci (QTLs) for late blight resistance have been identified (Ghislain *et al.*, 2001, Constanzo *et al.*, 2005, Mosquera, 2007). QTLs have been detected for *S. phureja* on chromosomes VII, XI, XII, in a cross between *S. phureja* x *S. tuberosum* (2n) (Ghislain *et al.*, 2001). A major resistance locus *Rpi-phu1* from *S. phureja*, conferring broad-spectrum resistance to late blight, was mapped to chromosome IX (Sliwka *et al.*, 2006). Two *PR-1* loci (*PR-1b1* and *PR-1b2)* which are supposed to play a role in horizontal resistance to LB in *S. phureja* were also located on chromosome IX (Evers *et al.*, 2006). Also, three major QTLs were identified on chromosomes III, V and XI in a cross between *S. phureja* x *S. stenotomum* (Costanzo *et al.*, 2005. Additionally, ten defense genes were clustered at a QTL on chromosome III and three defense genes were located at a QTL on chromosome XII (Trognitz *et al.*, 2002). On the other hand, QTLs with a negative effect on late blight resistance have also been reported in a cross between *S. phureja* varieties (Mosquera, 2007).

For the mapping of QTLs different types of marker have been used in potato, including RFLP, AFLP, SSR, SCAR and CAPs (Ghislain *et al.*, 2001, Constanzo *et al.*, 2005, Mosquera, 2007). Diversity Arrays Technology (DArT) is a hybridization-based technique, based on the amplification of a set of restriction fragments. Polymorphism in the restriction sites (SNPs or Indels) will result in the absence/presence of a particular fragment, which is detected by hybridization (Wittenberg, 2007). This marker system has been used for QTL mapping of disease resistance against powdery mildew and crown rot in wheat (Lillemo *et al.*, 2008, Ma *et al.*, 2010) and spot blotch in barley (Roy *et al.*, 2010). The identification of molecular markers linked to QTLs will allow using them in marker assisted selection, which will speed up the breeding process (Sliwka *et al.*, 2010) to obtain resistant varieties.

The aim of this research was to identify and map potential QTLs associated with LB resistance in a cross between two diploid potato landraces from Ecuador using DArT® markers.

Materials and methods

Plant materials

The progeny derived from a cross between two diploid *S. phureja* landraces from the germplasm collection known as Colección Ecuatoriana de la Papa (CEP) was used. Chaucha

amarilla (HSO-131), which is a moderately resistant potato landrace, was used as male parent, and the susceptible landrace Chaucha roja (HSO-369) was the female parent. The family obtained was designated as CHAR-01 and rendered 200 offspring plants of which 110 were deployed in a field experiment.

Field trial for late blight resistance

The field experiment was conducted in Quito at the Santa Catalina Experimental Station (EESC) of the National Institute for Agricultural Research (INIAP) located at 3050 m.a.s.l, Longitude: 78º33'15" and Latitude: 00º22'4" S. This location is known to have a high *P. infestans* pressure involving several complex races (Tello, 2008). Each individual of the progeny was planted in rows of 10 seeds each in a Complete randomized design. The plant material was evaluated under natural infection pressure for late blight resistance. The severity of the disease was assessed visually every 7 days for 4 weeks as percentage of necrotic leaf area. The evaluation started when the first symptoms were observed. The late blight severity values were used to calculate the Area Under the Disease Progress Curve (AUDPC) (Shanner & Finney, 1977):

$$AUDPC = \sum_{i=1}^{n} [(Y_{i+n1} + Y_i)/2] [X_{i+1} - X_i]$$

in which Y_i = late blight severity (per plot) at the i*th* observation, X_i = time (days) at the i*th* observation, and n = total number of observations. The late blight reaction of the clones were classified according their AUDPC values as: Highly Resistant (HR): <250; Resistant (R): 251-550; Moderately Resistant (MR): 551-650; Susceptible (S): 651-1200 and Highly Susceptible (HS): >1200 (Gopal & Singh, 2003). Broad sense heritability (h^2) was calculated as the ratio of the genetic variability (σ^2_g) to phenotypic variability ($\sigma^2_g + \sigma^2_e$) and is given by $h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_e)$. The estimation of the genetic and phenotypic variability was done according Singh *et al.* (1993).

Linkage map and QTL analysis

DNA samples of the CHAR-01 population were extracted according to the protocol provided Diversity Arrays Technology (DArT) Pty. Ltd (Yarralumla, Australia) and sent to them for analysis. A total of one thousand four hundred and twenty-six DArT® markers were used on the array in 110 individuals of the progeny. Each genotype was scored for presence (1) or absence (0) of a DArT marker. Linkage analysis was carried out using the software package JoinMap® 4.0 (Kyazma B.V., Wageningen, Netherlands). The linkage maps were constructed with the Cross Pollinated population type option and Haldane's mapping function linkage with a recombination threshold smaller than 0.40. The QTL analysis was conducted using MapQTL® 6.0 (Kyazma B.V., Wageningen, Netherlands). The phenotypic data used was the average of the AUDPC values of the field trial for each individual clone. A nonparametric test, single marker-based, Kruskal-Wallis analysis was used in a preliminary analysis to detect significant association of markers with AUPDC averages. Interval Mapping was then conducted in order to identify QTLs. To determine the significant genome-wise logarithm of the odds (LOD) threshold, a permutation test with 1.000 iterations was performed. A QTL was considered significant if it had a higher LOD score than the genome-wide threshold.

Results

Disease Phenotyping

A field experiment was conducted with offspring plants which were naturally infected by *P. infestans*. AUDPC values were obtained for one hundred ten individuals on the field. The average AUDPC was 1238.90. The minimum was 74.5 and the maximum was 3145. The coefficient of variation was 47.92%. The distribution of data was normal (W*= 0.98, P=0.43) (Figure 1). Most of the clones reacted as susceptible and highly susceptible, 91 out of 110. Meanwhile, 15 out of 110 reacted as moderately resistant to highly resistant (Table 1). The Broad Sense heritability (h^2) of the trait was 0.10.

Construction of the genetic linkage map using DArT markers and QTL Analysis

Using the 110 progeny plants and the 733 DArT markers which were polymorphic, 693 DArT markers were not polymorphic, a genetic linkage map for each of the parents was constructed. The parental map named P1 (Chaucha Amarilla) used 388 markers and the parental map P2 (Chaucha Roja) 134. Two hundred eleven markers were used in both maps as bridge markers. The map of the resistant parent (Chaucha amarilla, P1) covered 388.4 cM. The map was constructed with 248 DArT markers from a total of 599 markers. The map of the susceptible parent (Chaucha roja, P2) covered 262.4 cM and was based on 232 DArT markers from a total of 345. Chromosomes 8 and 12 had the lowest number of markers mapped, four and three respectively (Figure 2). In the parental map P2 no markers were associated to chromosomes 2, 3, 4, 8 and 12 (Figure 3).

The Kruskal-Wallis test was used to identify significant associations between the AUDPC data and the markers (Table 2). Nineteen such markers were identified. Thirteen markers were located on the chromosome VI, five markers on chromosome VII, one on chromosome I, all in the P1 map. Just one marker was significantly associated to a P2 marker, which was located on chromosome I. The permutation test was performed and LOD thresholds were calculated, which were 2.36 and 2.2 for P1 and P2 respectively. No QTLs were found since no peaks above the threshold were detected.

Discussion

Construction of linkage map

The mapping rendered an incomplete coverage of the genome. Some chromosomes had few markers associated. In the case of the P1 map Chromosomes 8 and 12 had the lowest number of markers associated (Figure 2). In the P2 map, chromosomes 2, 3, 4, 8 and 12 had no markers at all (Figure 3). This means that in order to definitively say something about the presence or absence of QTL against late blight more markers should be mapped so that also the missing chromosomes will contain DArT markers.

Segregation of Late blight resistance

Despite the variability observed in the AUDPC values in the CHAR-01 family, the fact that the heritability is extremely low (0.10) means that at this moment the environmental effects in the single location where the population was evaluated were too large. The fact that the population which was effectively scored for late blight resistance was relatively small (110 plants) and that the segregation of resistance was extremely skewed (the large majority >90% of the plants were susceptible) might play a role as well.

QTL mapping

No QTLs were identified in this population. A possible explanation of the failure is that the QTLs may have been located in regions poorly or not covered by markers in the linkage maps. QTLs had been reported in chromosome 12 in a population using a *S. phureja* accession from Ecuador (Ghislain *et al.*, 2001). In a population involving two *S. phureja* parents, QTLs were identified on chromosomes 2, 3, 4 and 9 (Mosquera, 2007). Another possibility is that several genes contributing to the late blight resistance phenotype in this segregating population were present and each with a small effect, so being non-significant for QTL detection. This later was supported by the low value of the heritability observed (Molina, 1992). Despite that some markers showed association with the trait the significance level was too low. Constanzo *et al.*, (2005) used as threshold criterion for QTL detection with Kruskal-Wallis test and a P-value of less than 0.01. Additionally, the association of the markers detected by this test pointed to chromosomes where no QTLs had been described before, so environmental effects could also serve as explanation for the variation in the late blight susceptibility observed in the field.

Another explanation for the absence of detection of QTLs in this population, could be attributed to the resistance level of the Ecuadorian potato landraces were most of them are susceptible. Additionally, those resistant potatoes are similar to the improved varieties (Monteros-Altamirano, 2011). The parental genotypes used for this progeny behaved not so different for their late blight reaction (Figure 1). Maybe field trials in more than one site would have been better for the detection of QTLs, if present. Further studies with more complete genome coverage will allow finding out if QTLs are present in this population and whether it will be worthwhile to try and introgress them into new cultivars.

Acknowledgments

To the Netherlands Organization for International Cooperation in Higher Education (NUFFIC), the Secretaria Nacional de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT), and the Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP) for the financial support.

Literature cited

Cañizares, C.A. , Forbes, G.A. 1995. Foliage resistance to *Phytophthora infestans* (Mont.) de Bary in the Ecuadorian national collection of *Solanum phureja* ssp.*phureja* Juz. & Buk Potato Research 38: 3-10.

Costanzo, S, Simko, I. Christ, B.J., Haynes, K.G. 2005. QTL analysis of late blight resistance in a diploid potato family of *Solanum phureja* x *S. stenotomum*. Theor Appl Genet 111: 609–617.

Cuesta, X.; Castillo, C.; Monteros, C. 2005. Biodiversidad de las papas nativas ecuatorianas. In Monteros, C.; Cuesta, X.; Jiménez, J.; López, G. (Eds.). Las papas nativas en el Ecuador. Quito, Ecuador, INIAP. Pp. 9-10.

Delgado, R. A., Monteros-Altamirano, A. R., Li, Y., Visser, R. G. F., van der Lee, T. A. J., Vosman, B. 2013. Large subclonal variation in *Phytophthora infestans* populations associated with Ecuadorian potato landraces. Plant Pathology, 62: 1081–1088. doi: 10.1111/ppa.12039

Diversity Arrays Technology Pty. Ltd. n.a. Plant DNA Extraction Protocol for DArT. Avaliable:http://www.diversityarrays.com/sites/default/files/pub/DArT_DNA_isolation.pdf. Date: 13/Sep/2011.

Escallon, R., Ramirez, M., Ñústez, C.E. 2005. Evaluación del potencial de rendimiento y de la resistencia a *Phytophthora infestans* (Mont. de Bary) en la colección de papas redondas amarillas de la especie *Solanum phureja* (Juz. *et* Buk.). Agronomía Colombiana 23 (1): 35-41.

Estrada, N. 2000. La biodiversidad en el mejoramiento genético de la papa. CIP-IPGRI-PRACIBA-IBTA-PROINPA-COSUDE-CID, La Paz, Bolivia.

Evers D., Schweitzer C., Nicot N., Gigliotti S., Herrera M.R., Hausman J.F., Hoffmann L. Trognitz B., Dommes J., and Ghislain M. 2006. Two PR-1 loci detected in the native cultivated potato *Solanum phureja* appear differentially expressed upon challenge by late blight. Physiological and Molecular Plant Pathology 67: 155–163.

Forbes GA, Escobar XC, Ayala CC, Revelo J, Ordoñez ME, Fry BA, Doucett K, Fry WE. 1997. Population Genetic Structure of Phytophthora infestans in Ecuador. Phytopathology. 1997 Apr;87(4):375-80.

Gabriel, J., Plata G; Cadima X.; Franco J. 2013. *Solanum phureja* Juz et Buk.: Valuable Source of Genetic Resistance to Potato Late Blight [*Phytophthora infestans* (Mont.) de Bary]. Revista Latinoamericana de la Papa 17 (2):131- 142.

Garofalo, J. 2005. Evaluación de la aptitud combinatoria general y específica en 21 progenies de papa S*olanum phureja* para resistencia a tizón tardío P*hytophthora infestans.* Tesis Ing. Agrónomo. Quito, Universidad Central del Ecuador. 123p.

Ghislain, M., Trognitz, B.,· Herrera, Ma. del R., Solis, J, Casallo, G., Vásquez, C., Hurtado, O., Castillo, R., Portal, L., Orrillo, M. 2001. Genetic loci associated with field resistance to late blight in offspring of *Solanum phureja* and *S. tuberosum* grown under short-day conditions Theor Appl Genet (2001) 103:433–442.

Gopal, J., Singh, B.P. 2003. Screening potatoes for resistance to late blight (*Phytophthora infestans*) under field conditions. *Potato Res* **46**: 47-56.

Lillemo, M., Asalf, B., Singh, R. P., Huerta-Espino, J., Chen, X. M., He, Z. H., Bjørnstad, Å. 2010. The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar Theor Appl Genet. 120:1119–1128.

Ma, J. Li, H. B., Zhang, C. Y., Yang, X. M., Liu, Y. X., Yan, G. J. Liu, C. J. 2010. Identification and validation of a major QTL conferring crown rot resistance in hexaploid wheat Theor Appl Genet 120:1119–1128.

Molina, J.D. 1992. Introducción a la genética de poblaciones y cuantitativa. A.G.T. Editor, Mexico.

Monteros-Altamirano A, 2011. *Potato landraces: description and dynamics in three areas of Ecuador.* Wageningen, The Netherlands: Wageningen University, PhD Thesis.

Monteros, C.; Reinoso, I. 2010. Biodiversidad y oportunidades de mercados para las papas nativas ecuatorianas. In Memorias XXIV Congreso Latinoamericano de la Asociación Latinoamericana de la Papa. Cusco, Perú, INIA. Pp. 447-448.

Morales, R., Revelo, J., Suarez, G., Forbes, G., Muñoz, F. 1995. Relación entre el ataque de *Phytophthora infestans* y la producción en el cultivo de la papa (*Solanum tuberosum*). In Memorias XVII Reunión de la Asociación Latinoamericana de la Papa. Mérida, Venezuela.

Mosquera, T. 2007. Análisis genético y molecular de la resistencia cuantitativa a tizón tardío (*Phytophthora infestans*) en *Solanum phureja*. PhD Thesis, Universidad Nacional de Colombia.

Oyarzún, P.; Gallegos, P.; Asaquibay, C.; Forbes, G.; Ochoa, J.; Paucar, B.; Prado, M.; Revelo, J.; Sherwood, S.; Yumisaca, F. 2002. Manejo integrado de plagas y enfermedades. *In* Pumisacho, M.; Sherwood, S. El cultivo de la papa en Ecuador. Quito, INIAP/CIP. Pp. 85-169.

Revelo J., Garcés S., Andrade J. 1997. Identification of sources of horizontal resistance to *Phytophtora infestans* free of major genes. CIP Circular (April): 15-17.

Roy, J.K., Smith, K.P., Muehlbauer, G.J., Chao, S., Close, T.J., Steffenson, B.J. 2010. Association mapping of spot blotch resistance in wild barley. Mol Breeding 26:243–256.

Shanner G., Finney R.E. 1977. The Effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67: 1051-1056.

Singh, J. Ceccarelli, S. Hamblin, J. 1993. Estimation of heritability form variety field trials data. Theoretical and Applied Genetics 86: 437-441.

Sliwka J, Jakuczun H, Lebecka R, Marczewski W, Gebhardt, Zimnoch-Guzowska E, 2006. The novel, major locus *Rpi-phu1* for late blight resistance maps to potato chromosome IX and is not correlated with long vegetation period. Theor Appl Genet 113: 685–695.

Tello C. 2008. Identificación de aspectos epidemiológicos relacionados con la expresión de resistencia de la papa (*Solanum tuberosum*) para las poblaciones de *Phytophthora infestans* predominantes en tres localidades de la sierra ecuatoriana. Tesis Ing. Agr. Universidad Central del Ecuador: 95.

Thurston H.D., Schultz, O. 1981. Late blight. In Hooker, W.J. (ed.) Compendium of potato diseases. St. Paul, U.S.: American Phytopathological Society, 40 – 42.

Trognitz F., Manosalva P., Gysin R., Niño-Liu D., Simon R., Herrera M. R., Trognitz B., Ghislain M., and Nelson R. 2002. Plant Defense Genes Associated with Quantitative Resistance to Potato Late Blight in *Solanum phureja* × Dihaploid *S. tuberosum* Hybrids. MPMI 15 (6): 587–597.

Wickramasinghe, W. M. D. K., Qu, X.S., Costanzo, S., Haynes, K.G., Christ, B.J. 2009. Development of PCR-based markers linked to quantitative resistance to late blight in a diploid hybrid potato population of *Solanum phureja* x *S. stenotomum*. Am. J. Pot Res (2009) 86:188–195.

Wittenberg, A.H.J. 2007. Genetic mapping using the Diversity Arrays Technology (DArT) - Application and validation using the whole-genome sequences of *Arabidopsis thaliana* and the fungal wheat pathogen *Mycosphaerella graminicola* PhD thesis, Wageningen University, The Netherlands.

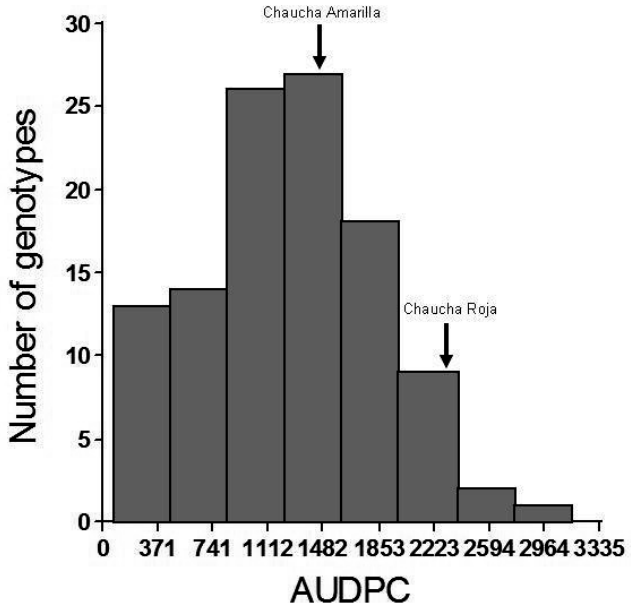


Figure 1. Distribution by mean AUDPC of 110 clones from the diploid family CHAR-01. Parentals are indicated with arrows.

Table 1. Reaction to late blight of 110 clones from the diploid family CHAR-01 according to AUDPC values (Gopal & Sigh , 2003).

Late blight reaction*	Genotype	Total
HR	142, 530, 556, 100, 7	5
R	152, 233, 109, 4, 84, 140, 6, 52, 18, 726	10
MR	31, 113, 514, 237	4
S	20, 15, 38, 185, 1, 101, 195, 518, 548, 205, 14, 220, 110, 85, 34, 529, 130, 262, 114, 708, 559, 403, 96, 106, 206, 549, 257, 88, 42, 119, 700, 170, 10	33
HS	3, 527, 266, 211, 28, 128, 45, 55, 53, 76, 74, 60, 41, 172, 73, 68, 36, 512, 75, 153, 397, 32, 525, 555, 93, 537, 545, 125, 533, 24, 126, 546, 118, 56, 122, 98, 507, 70, 513, 242, 508, 524, 17, 104, 528, 522, 521, 16, 19, 39, 8, 750, 308, 502, 511, 509, 131, 5	58

*HR (Highly Resistant): <250; R (Resistant): 251-550; MR (Moderately Resistant): 551-650;
S (Susceptible): 651-1200; HS (Highly Susceptible): >1200.

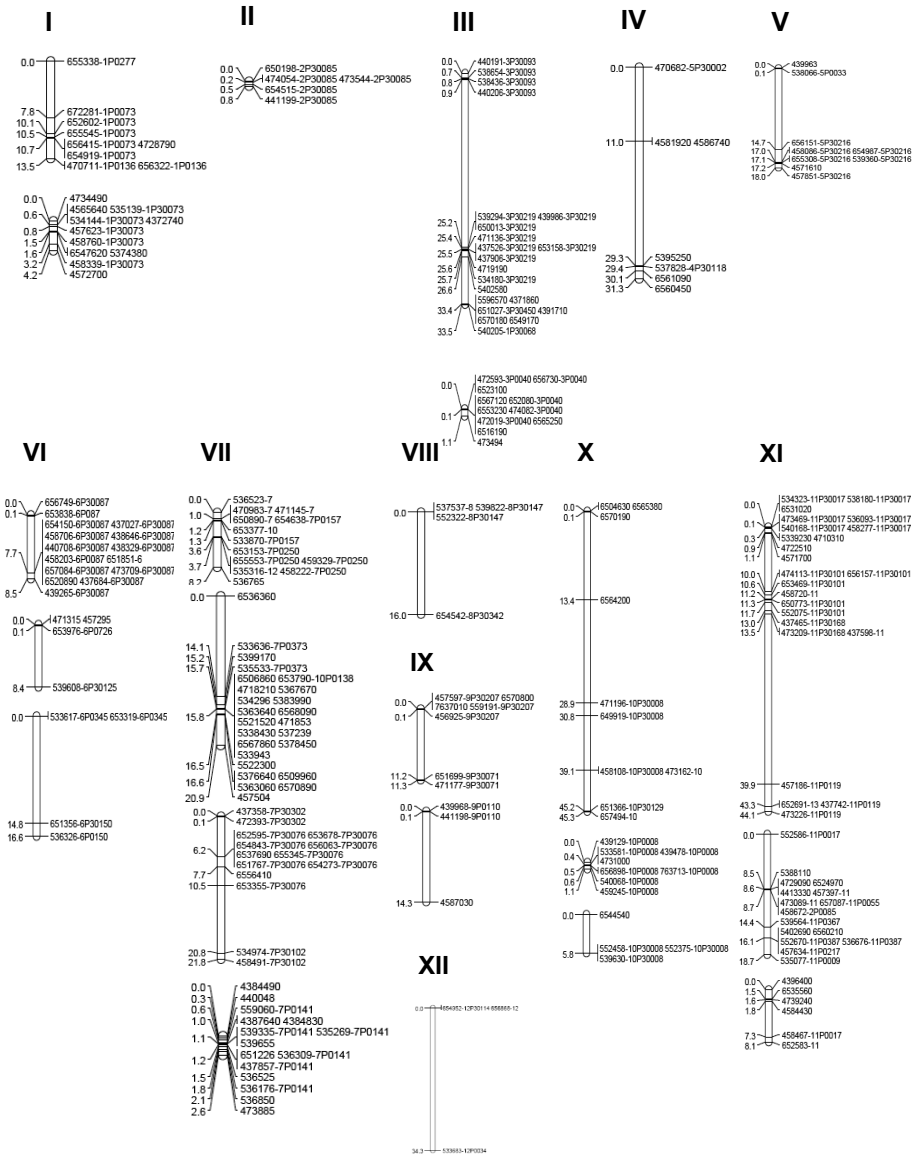


Figure 2. Linkage map of the Parental Type P1 (Chaucha amarilla).

Figure 3. Linkage map of the Parental Type P2 (Chaucha roja). Note for chromosomes 2,3,4,8 and 12 no markers were found

Table 2. Markers associated to late blight resistance according to Kruskal-Wallis test.

Parental type	Chromosome	Marker	K*	Signif.
P1	VI	657084-6P30087	7.50	0.01
P1	VI	6520890	7.50	0.01
P1	VI	458706-6P30087	6.23	0.05
P1	VI	440708-6P30087	6.23	0.05
P1	VI	654150-6P30087	6.20	0.05
P1	VI	438646-6P30087	6.20	0.05
P1	VI	473709-6P30087	6.17	0.05
P1	VI	437684-6P30087	6.17	0.05
P1	VI	458203-6P0087	5.96	0.05
P1	VI	437027-6P30087	5.93	0.05
P1	VI	439265-6P30087	5.92	0.05
P1	VI	651851-6	5.91	0.05
P1	VI	438329-6P30087	5.85	0.05
P1	VII	539655	4.87	0.05
P1	VII	654273-7P30076	4.25	0.05
P1	VII	536850	4.08	0.05
P1	VII	6556410	4.07	0.05
P1	VII	654843-7P30076	4.02	0.05
P1	I	672281-1P0073	3.92	0.05
P2	I	672281-1P0073	3.92	0.05

Chapter 7

Late blight response and yield characteristics of potato varieties in two environments in Chimborazo province, Ecuador

R.A. Delgado[1,2,3], J. Rivadeneira[1], X. Cuesta[1], S. Silva[1], J Vossen[2], D.Danial[2], R.G.F. Visser[2].

[1] Instituto Nacional Autónomo de Investigaciones Agropecuárias (INIAP), Estación Experimental Santa Catalina, Panamericana Sur km 1, Quito, Ecuador.
[2] Plant Breeding, Wageningen University and Research, P.O. Box 16, 6700 AA, Wageningen, The Netherlands.
[3] Graduate school Experimental Plant Sciences, Wageningen University

Abstract

The late blight susceptibility and yield of fifteen potato varieties was evaluated in two locations in Ecuador, Tunshi and Quimiag in Chimborazo province. There were significant differences observed for environments, genotypes and the interaction between these two. In Tunshi, clones Carolina and 00_24_1 were shown to be highly resistant, while Superchola, 99_66_6 and 08_12_01 were resistant to late blight. In Quimiag, Carolina, 99_66_6, INIAP-Estela and INIAP-Natividad were resistant to the disease. For yield per hectare, Carolina (34.6 t/ha), 08_12_1 (28.67 t/ha), INIAP-Estela (27.63 t/ha), 97_25_3 (26.6 t/ha), INIAP-Natividad (26.33 t/ha), Superchola (22.27 t/ha), 08_12_2 (21.5 t/ha) and 00_24_1 (20.3 t/ha) were the most productive. In Quimiag, Carolina (14.4 t/ha), INIAP-Estela (13.3 t/ha) and INIAP-Natividad (12.3 t/ha) had the highest yields for that site. In Tunshi, non-significant correlations among yield and late blight assessments and AUDPC were observed. On the other hand, in Quimiag yield was negatively correlated to AUDPC and late blight severity assessments. Despite the variation observed among the two sites, Carolina and 99_66_6, were the best performing clones with regard to late blight resistance. Carolina, INIAP-Estela and INIAP-Natividad were those that had the higher yields in both places. The variability observed shows the difficulties for breeding potatoes in Ecuador. Nevertheless, the varieties that performed good in both places are valuable for breeding and should be used as parental clones in the country.

Keywords: Breeding, *Phytophthora infestans*, *Solanum*, disease resistance

Introduction

In Ecuador potatoes are an important staple crop and challenged mainly by late blight. One of the main constraints for tuber yield is the occurrence of late blight epidemics caused by the oomycete *Phytophthora infestans* (Oyarzún *et al.*, 2002b). It causes necrosis of the leaves and all other organs of the plant thus reducing harvestable potato yield to zero in severe cases (Thurston & Schultz, 1981;Morales *et al.*, 1995). Conditions in the Ecuadorian highlands are favourable for late blight epidemics because of the highly favourable temperatures ranging from 12 to 18 ^{o}C, the high humidity, and potato being grown all year around (Oyarzún *et al.*, 2002b).

The epidemic starts from the sporangia grown on the surface of the infected tissue, which can be dispersed by rain and wind. Once landed on a susceptible tissue, sporangia can germinate producing mycelia or release zoospores both capable to infect the host. The pathogen can survive in infected tubers, plant debris and volunteer plants. Infection of tubers can occur but is rarely observed (Oyarzún *et al.*, 2005, Kromman *et al.,* 2008), or even absent (Fankhauser, 2000) when tuber diseases had been prospected. These have been attributed to soil supressiveness due to physical/chemical properties (Oyarzun *et al.*, 2011, Villamarin *et al.*, 2011) and/or microbial antagonism (Orquera *et al.*, 2011). Late blight epidemics could start soon after the emergence through infected sprouts from zoospores or sporangia from infected potato plants in the field (Kromman *et al.,* 2008). Despite the fact that the population of *P. infestans* belongs to the A1 mating type, so it reproduces clonally only, it has been observed that it is composed of complex races capable to overcome several major resistant genes (Forbes *et al.*, 1997, Tello, 2008, Chapter 3; Delgado *et al.*, 2013). It has also been observed that this asexual reproducing population has different genotypes among them based on microsatellite analyses (Chapter 3; Delgado *et al.*, 2013). Particularly, taken into consideration the race composition, in Chimborazo a high diversity has been observed with an Evenness near a value of 1, which means that almost every isolate analyzed was a unique race phenotype (Chapter 3; Delgado *et al.*, 2013).

For this reason, breeding efforts are continuously undertaken in order to obtain resistant varieties. Variability in the late blight development on advanced breeding clones and in the improved varieties has been observed in the different evaluation sites in Ecuador (Rivadeneira *et al.*, 2008). Also, Forbes *et al.*, (2005) observed variation in the late blight development on a group of potato varieties planted in different countries. They attributed this to the variability of *P. infestans* populations in Argentina, where both mating types occur and where sexual recombination results in an increased genetic variation. Also the epidemiological conditions in Ecuador, where infections could occur soon after emergence of the sprouts, since inoculum sources are available throughout the year, may give the chance for spontaneous mutations to occur. Additionally, a change in the population of the pathogen in Ecuador from predominantly simple to highly complex races, capable to overcome several major resistance genes, has been reported (Forbes *et al.,* 1997, Delgado *et al.*, 2013).

The aim of this study was to analyse the resistance to late blight of a set of fifteen potato cultivars at two locations in Chimborazo province in Ecuador with the aim to determine if they behave similarly under different conditions. Correlations between late blight resistance, tuber yield and location are studied.

Materials and methods

Testing site

The present research was carried out in two locations in the Chimborazo province, with two different rainfall conditions, which means that one location (Quimiag) is probably more conductive for late blight epidemics. Quimiag (2834 meters above sea level [masl], 1000 mm average rainfall/year, 12 °C average/year, 75% average relative humidity, 1° 40'42'' longitude, 78°38'24'' latitude) and Tunshi (2829 masl, 421 mm average rainfall/year, 13.8 °C average/year, 66.4% average relative humidity,1º 44' 36'' longitude, 78º 37' 33'' latitude). Nutrient contents of the soil at both sides proved to be different (Table S1).

Testing materials and treatments

The experiment consisted of three replications (plots) placed in each of the two locations in Chimborazo province. Fifteen potato clones/cultivars, nine of them selected from the breeding lines of INIAP's breeding program, all obtained from crosses using Ecuadorian potato landraces , and four Ecuadorian varieties and two clones, one from Colombia and one from Perú (Table 1) were used. These clones were included in each plot according to a randomized block design, the examined materials consisted of nine advanced clones which have been developed in Ecuador and six commercial varieties that were developed locally or introduced. The seed tubers were obtained from Santa Catalina Research Station in Quito, Pichincha Province. They were treated with Carboxin (100 mL/L) before planting. Plots measured 12 m^2 with 4 rows, each of them had 3 m of length and 30 cm distance between plants and 1 m distance between rows. Fertilization was applied according to crop requirements based on nutrient contents prior to planting adding Nitrogen, Phosphorus and Potasium, in each row (Table S1, Oyarzun *et al.*, 2002a).

Planting took place on November 17th, 2011 in Quimiag and October 24th, 2011 in Tunshi. Plants were earthed up 64 and 75 days after planting in Quimiag and Tunshi, respectively. Profenopos (2.5 ml/l) was applied in order to control the potato flea beetle (*Epitrix* sp.) in both sites. One application of contact fungicide (Mancozeb, 2,25 kg/ha) was done 30 days after emergence of the sprouts in order to protect the plants from early infection by *P. infestans*, after this, natural infection by the pathogen was allowed. Disease severity was recorded as percentage of necrotic tissue.

Assessment of late blight resistance

The evaluations of late blight started 46 and 69 days after planting in Quimiag and Tunshi, respectively and were registered weekly. The late blight severity values were used to calculate the Area Under the Disease Progress Curve (AUDPC) according to Shanner & Finney (1977):

$$AUDPC = \sum_{i=1}^{n} [(Y_{i+n1} + Y_i)/2] [X_{i+1} - X_i]$$

in which Y_i = late blight severity (per plot) at the i*th* observation, X_i = time (days) at the i*th* observation, and n = total number of observations.

Yield assessments

At harvest, tubers were collected from each plot and weighed. Data were extrapolated to tons per hectare.

Data of AUDPC and Yield were subjected to analysis of variance (ANOVA) for each environment in order to asses differences among the genotypes and means were compared by Scott & Knot test (α= 0.05) (Gates & Bilbro, 1978). A combined ANOVA was performed for both environments to check genotype – environment interaction. The heritability (h^2) of AUDPC and Yield per hectare was calculated according Singh *et al.* (1993) by the formula: $h^2 = \sigma^2_{g/} [\sigma 2g_+ \sigma^2_{i+} \sigma^2_e]$, where σ^2_e = MSE, σ^2_i = (MSI – MSE)/b and, σ^2_g = (MSG – MSI)/bl. MSE stand for Mean Square of Error, MSI for Mean Square of Interaction, MSG for Mean Square of Genotypes, b for number of replications and l for number of locations.

The correlations between AUDPC, Yield and severity assessments in each site were estimated with the Spearman's coefficient (Bonierbale *et al.*, 2006).

The cultivars were classified by their susceptibility to late blight based their average AUDPC values in each location according the following scale: highly resistant (< 250), resistant (251-550), moderately resistant (551-650), susceptible (651-1200) and highly susceptible (>1200) (Gopal & Singh, 2003).

Results and Discussion

The single ANOVA performed for each environment resulted in a significant effect of genotypes for AUDPC and yield. For both variables, the better performance was registered in Tunshi with lower mean AUDPC (504.04) and higher mean yield (20.07 ton/ha) than in Quimiag with 1236.44 (AUDPC) and 7.28 ton/ha, respectively (Table 2). The results are in agreement with different weather conditions in both locations, being more favourable for late blight epidemics in Quimiag with high rainfall than in Tunshi.

In the combined ANOVA we observed significant differences for environments, genotypes and the interaction (G x E) (Table 3). It was observed that yield per hectare had a heritability (h^2) of 0.44 while it was 0.43 for AUDPC. The heritability values are low indicating that the traits observed were largely influenced by the environment. Similar and higher heritability values for late blight resistance have been reported by Landeo *et al.*, (1999) (0.40-0.53), Costanzo *et al.*, (2004) (0.67) and Pinto *et al.*, (2002) (0.87-0.95). For yield per hectare, higher heritability values had been reported by Perez-Lopez *et al.*, (2007) (0.79-0.90).

In Tunshi, it was observed that the clones Carolina and 00_24_1 were highly resistant, Superchola, 99_66_6 and 08_12_01 were resistant to late blight. In Quimiag, Carolina, 99_66_6, INIAP-Estela and INIAP-Natividad were resistant to the disease (Table 4). In both sites Carolina and 99_66_6 were resistant. The variation on the late blight reaction (Table 4) between sites may be due to the weather conditions that are more humid in Quimiag than in

Tunshi. Other conditions that may influence the reaction of the potatoes planted in this trial maybe that potatoes are grown all year around, so inoculum is available for early infection (Forbes *et al.*, 2005), especially in Quimiag where the climate is more favourable for late blight epidemics. Additionally, the pathogen population in each location is most probably different and may explain the differences in the behaviour of the potato varieties in both locations. Recently it has been observed that the population of *P. infestans* in Chimborazo, despite the fact that is part of a clonal lineage (EC-1), has diversity in races and even in genotypes (Chapter 3; Delgado *et al.*, 2013). So the different behaviour of the potato varieties may also be influenced by differences in the populations of the pathogen in each location. These may be the case of Superchola which in Tunshi behaved as resistant but was susceptible in Quimiag. Similarly, INIAP-Estela and INIAP-Natividad were susceptible in Tunshi but resistant in Quimiag.

For Yield per hectare, Carolina (34.6 t/ha), 08_12_1 (28.67 t/ha), INIAP-Estela (27.63 t/ha), 97_25_3 (26.6 t/ha), INIAP-Natividad (26.33 t/ha), Superchola (22.27 t/ha), 08_12_2 (21.5 t/ha) and 00_24_1 (20.3 t/ha) were the most productive. In Quimiag, Carolina (14.4 t/ha), INIAP-Estela (13.3 t/ha) and INIAP-Natividad (12.3 t/ha) had the highest yields for that site (Table 4).

In Tunshi, non-significant correlations among yield and late blight assessments and AUDPC were observed (Table 5). This may be due to the low average rainfall that occurs in that site. AUDPC was highly correlated with late blight severity in 97 DAP (0.95, $\alpha < 0.001$) (Table 5.) Meanwhile in Quimiag, yield was negatively correlated to AUDPC and late blight severity assessments starting from 67 until the last one, 94 days after planting, with 80 DAP with the highest correlation (-0.71, $\alpha < 0.001$). The highest correlation between AUDPC and severity assessment in Quimiag was with 80 DAP (0.99, $\alpha < 0.001$) (Table 6). This situation in Quimiag can be attributed due to the higher average rainfall that occurs in that location, so epidemics have favourable conditions, resulting in a negative correlation among late blight and yield.

Consequences for the breeding program

The variation in the late blight resistance and yield observed in this research points out the difficulties for potato breeding in Ecuadorian highlands. Two evaluation sites in the same province led already to quite different behaviour of the varieties. Nevertheless, some varieties performed good in both sites for late blight resistance, which was the case of Carolina and 99_66_6, while others were superior just in one of the locations. For yield, Carolina, INIAP-Estela and INIAP-Natividad were in both sites superior. This behaviour may serve as an indication of the adaptation of these varieties to different environments. These characteristics made these clones valuable for breeding, so they should be used as progenitors for future development of new varieties with late blight resistance and high yield.

Acknowledgements

We thank the Netherlands Organization for International Cooperation in Higher Education (NUFFIC), the Secretaría Nacional de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT) – Ecuador and the Instituto Nacional Autónomo de Investigaciones Agropecuárias (INIAP) – Ecuador, for financial support.

Literature Cited

Bonierbale M, Haan S, Forbes A. 2006. Procedures for standard evaluation trials of advanced potato clones. An International Cooperators' Guide. Lima, Perú: International Potato Center (CIP).

Costanzo S, Christ B. J., Haynes K. G. 2004. Late blight resistance in a diploid full-sib potato family. Plant Breeding 123, 377—381.

Delgado, R. A., Monteros-Altamirano, A. R., Li, Y., Visser, R. G. F., van der Lee, T. A. J., Vosman, B. 2013. Large subclonal variation in *Phytophthora infestans* populations associated with Ecuadorian potato landraces. Plant Pathology, 62: 1081–1088. doi: 10.1111/ppa.12039

Fankhauser C. 2000. Seed-transmitted diseases as constraints for potato production in the tropical highlands of Ecuador. Zurich, Switzerland: Swiss Federal Institute of Technology Zurich, PhD thesis.

Forbes GA, Escobar XC, Ayala CC, Revelo J, Ordoñez ME, Fry BA, Doucett K, Fry WE, 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. *Phytopathology* 87, 375-380.

Forbes, G. A., Chacón, M. G., Kirk, H. G., Huarte, M. A., Van Damme, M., Distel, S., Mackay, G. R., Stewart, H. E., Lowe, R., Duncan, J. M., Mayton, H. S., Fry, W. E., Andrivon, D., Ellissèche, D., Pellé, R., Platt, H. W., MacKenzie, G., Tarn, T. R., Colon, L. T., Budding, D. J., Lozoya-Saldaña, H., Hernandez-Vilchis, A., Capezio, S. 2005. Stability of resistance to *Phytophthora infestans* in potato: an international evaluation. Plant Pathology, 54: 364–372. doi: 10.1111/j.1365-3059.2005.01187.x

Gates, C.E., Bilbro, J.D. 1978. Illustration of a cluster analysis method for mean separation. Agronomy Journal 70: 462–465.

Gopal, J., Singh, B.P. 2003. Screening potatoes for resistance to late blight (*Phytophthora infestans*) under field conditions. *Potato Res* **46**: 47-56.

Kromann, P., Taipe, A., Andrade-Piedra, J. L., Munk, L., Forbes, G. A. 2008. Preemergence infection of potato sprouts by *Phytophthora infestans* in the highland tropics of Ecuador. Plant Dis. 92:569-574.

Landeo J A, Gastelo M, Beltran G, Diaz L. 1999. Quantifying genetic variance for horizontal resistance to late blight in potato breeding population B3C1. CIP Program Report 1999-2000. Pp. 63 – 68.

Oyarzún P, Chamorro F, Cordova J, Merino F, Valverde F, Velasquez J. 2002a. Manejo agronomico. In: Pumisacho M, Sherwood S, eds. *El Cultivo de la Papa en Ecuador*. Quito, Ecuador: INIAP/CIP, 51–83.

Oyarzún P, Gallegos P, Asaquibay C, Forbes G, Ochoa J, Paucar B, Prado M, Revelo J, Sherwood S, Yumisaca F. 2002b. Manejo integrado de plagas y enfermedades. In: Pumisacho M, Sherwood S, eds. *El Cultivo de la Papa en Ecuador*. Quito, Ecuador: INIAP/CIP, 85–169.

Oyarzún PJ, Garzón CD, León D, Andrade I, Forbes GA. 2005. Incidence of Potato Tuber Blight in Ecuador. Amer J of Potato Res 82:117-122.

Oyarzún PJ, Krijger AK, Garzón CD, Leon D, Kromann P, Yuen JE, Forbes, GA. (2011). Evaluation of host susceptibility, pathogen aggressiveness and sporangial survival in soil as factors affecting incidence of potato tuber infection by *Phytophthora infestans* in Ecuador. *Tropical Plant Pathology*, *36*(3), 141-149.

Pérez-López D. J., Vázquez-García L. M., Sahagún-Castellanos J., Rivera-Peña A. 2007. Variabilidad y caracterización de diez variedades de papa en tres localidades del Estado de México. Revista Chapingo Serie Horticultura 13(1): 13-19, 2007.

Pinto C.A.B.P., Faria C.A., Lambert E.S. 2002. Potato clones resistance to early and late blight. Crop Breeding and Applied Biotechnology 2(2),189-196.

Rivadeneira J., Tello., C., Cuesta X., Ochoa J., Reinoso I. 2008. Aspectos epidemiológicos relacionados con la expresión de la resistencia al tizón tardío en papa. In III Congreso Iberoamericano de Investigación y Desarrollo en Patata. 5 al 10 de Octubre, 2008, Vitoria-Gasteiz, Euskadi, España. Pp. 171-173.

Shaner G., Finney R.E. 1977. The Effect of Nitrogen Fertilization on the Expression of Slow-Mildewing Resistance in Knox Wheat. Phytopathology 67: 1051-1056.

Singh, J. Ceccarelli, S. Hamblin, J. 1993. Estimation of heritability form variety field trials data. Theoretical and Applied Genetics 86: 437-441.

Orquera G, Mogrovejo C, Villamarín D, Jarrin F, Garzon CD, Molineros J, Forbes GA, Koch A, Benítez M. 2011. Characterization of microbial populations from *P. infestans* suppressive Andean soils in Ecuador. Phytopathology 101:S133

Torres, L., Cuesta, X., Monteros, C., Rivadeneira, J. 2011. Variedades. In: J. Andrade-Piedra & L. Torres (eds.). Inventario de Tecnologías e Información para el Cultivo de Papa en Ecuador (en línea).[http://cipotato.org/region-quito/informacion/inventario-de-tecnologias/variedades] Accessed April 28, 2013.

Villamarín D, Orquera G, Mogrovejo C, Garzon CD, Molineros J, Forbes GA, Koch A, Benitez M. 2011. Suppressiveness to *Phytophthora infestans* infection in potato tubers by Andean soils from three provinces of Ecuador. Phytopathology 101:S183

Table 1. Potato genotypes used in the present research.

Genotypes	Pedigree	Category	Origen[1]	Skin color	Days to maturity
99-32-1	B1C41103 x Margarita	Advanced clone	INIAP-Ecuador	Cream	130-150
07-46-8	05-18-5 x Estela	Advanced clone	INIAP-Ecuador	Red	130-150
97-25-3	B1 x Fripapa	Advanced clone	INIAP-Ecuador	red	130-150
07-29-18	ASO861*HSO213	Advanced clone	INIAP-Ecuador	red	130-150
08-12-1	BOM-532 X I-Fripapa	Advanced clone	INIAP-Ecuador	red	130-150
08-12-2	BOM-532 X I-Fripapa	Advanced clone	INIAP-Ecuador	Red	130-150
08-12-4	BOM-532 X I-Fripapa	Advanced clone	INIAP-Ecuador	Red	130-150
99-66-6	Fripapa x B2C3075	Advanced clone	INIAP-Ecuador	Red	130-150
00-24-1	Uvilla x D-134	Advanced clone	INIAP-Ecuador	cream	130-150
Superchola	(Curipamba negra x *Solanum demissum)* x (resistant clone with yellow flesh x Chola)	Variety	Private Breeder-Ecuador	pink	180
INIAP-Natividad	(INIAP-Gabriela x (*Solanum phureja* x *Solanum pausissectum*)	Variety	INIAP-Ecuador	Cream/pink	145-170
INIAP-Fripapa	(Bulk México x 378158.721) x I-1039	Variety	INIAP-Ecuador	pink	180
INIAP-Estela	Superchola x (*Solanum phureja* x *Solanum pausissectum*)	Variety	INIAP-Ecuador	purple	145 -160
Carolina	38139.16 x I – 039	Variety	CIP-Perú	cream	90-120
Capiro	Tuquerreña (CCC 61) x 1967 (C) (9) (CCC751).	Variety	ICA-Colombia	Red	165

[1] INIAP: Instituto Nacional Autónomo de Investigaciones Agropecuárias, CIP: Centro Internacional de la Papa, ICA: Instituto Colombiano Agropecuario.

Table 2. Means Squares of individual analysis of variance of 15 genotypes in two environments in Chimborazo province, Ecuador.

Source of Variation	Degrees of freedom	AUDPC				Yield (ton/ha)			
		Tunshi		Quimiag		Tunshi		Quimiag	
Block	2	4131.09	n.s.	17166.96	n.s.	20.47	n.s.	0.4	n.s.
Genotype	14	191765.95	***	1491005.03	***	172.1	***	43.21	***
Error	28	2282.66		14872.38		37.59		5.75	
Mean		504.04		1236.44		20.07		7.28	
CV (%)		9.48		9.86		30.55		32.93	

*** Significant at $\alpha < 0.001$, n.s. non-significant.

Table 3. Mean Squares of combined analysis of variance of AUDPC and Yield of fifteen potato genoptypes in two environments in Chimborazo province, Ecuador.

Source of Variation	Degrees of freedom	Yield (ton/ha)		AUDPC	
Block	2	7.66	n.s.	9552.34	n.s.
Environments (E)	1	3678.72	***	12069219.6	***
Genotypes (G)	14	173.74	***	1209975.76	***
G x E	14	41.58	*	472795.22	***
Error	58	21.37		8686.77	
Mean		13.67		870.24	
CV (%)		33.81		10.71	

* Significant at α< 0.05, *** Significant at α< 0.001, n.s. non-significant.

Table 4. Mean of AUDPC, Yield (ton/ha) and Late blight (LB) reaction of fifteen potato genotypes in two locations of Chimborazo province, Ecuador.

Genotypes	TUNSHI			QUIMIAG		
	AUDPC	LB reaction	Yield	AUDPC	LB reaction	Yield
Carolina	169 A	HR	34.6 A	416 A	MR	14.4 A
00_24_1	228 A	HR	20.3 A	1032.67 C	S	7.7 C
Superchola	255.67 A	R	22.27 A	996.67 C	S	5 C
99_66_6	276.33 A	R	13.17 B	656 A	S	4.1 C
08_12_1	308.67 B	R	28.67 A	830 B	S	9.5 B
INIAP-Fripapa	385.67 B	MR	10.8 B	1814.33 E	HS	3.4 C
99_32_1	389.33 B	MR	11.9 B	1504 D	HS	4.8 C
INIAP-Estela	507.33 C	MR	27.63 A	422.67 A	R	13.3 A
07_46_8	562 C	MR	15.37 B	830.33 B	S	9.1 B
INIAP-Natividad	564.67 C	MR	26.33 A	545 A	MR	12.3 A
97_25_3	585 C	MR	26.6 A	1168.33 C	S	4.8 C
08_12_2	587.33 C	MR	21.5 A	1437.67 D	HS	5.9 C
Capiro	880.33 D	S	10.8 B	2902.67 F	HS	1.3 C
07_29_18	904 D	S	13.2 B	2005.67 E	HS	6.4 C
08_12_4	957.33 D	S	17.53 B	1984.67 E	HS	7.2 C
Mean	504.04		20.07	1236.44		7
CV (%)	9.48		30.55	9.86		33

Means followed by the same letter are not significantly different (α=0.05) according to Scott & Knott test.
HR= Highly resistant, R= Resistant, MR= Moderately resistant, S= Susceptible, HS= Highly susceptible.

Table 5. Spearman's correlation coefficients between AUDPC, Yield and severity evaluations in Tunshi, Chimborazo province, Ecuador.

	69dap	76dap	83dap	90dap	97dap	104dap	AUDPC
69dap							
76dap	0.44 ns						
83dap	0.63**	0.58 **					
90dap	0.37 ns	0.5 ns	0.55 *				
97dap	0.38 ns	0.67 *	0.61 *	0.86 **			
104dap	0.33 ns	0.52 ns	0.31 ns	0.84 *	0.83 *		
AUDPC	0.33 ns	0.65 *	0.46 ns	0.92 ***	0.95 ***	0.91 ***	
Yield	0.21 ns	-0.08 ns	-0.11 ns	-0.21 ns	-0.31 ns	-0.26 ns	-0.29 ns

Data significant at $\alpha < 0.05$ (*), $\alpha < 0.01$ (**), $\alpha < 0.001$ (***).

Table 6. Spearman's correlation coefficients between AUDPC, Yield and severity evaluations in Quimiag Chimborazo province, Ecuador.

	46dap	53dap	60dap	67dap	73dap	80dap	87dap	94dap	AUDPC
46dap									
53dap	0.73 *								
60dap	0.52 ns	0.72 *							
67dap	0.56 *	0.67 *	0.75 *						
73dap	0.34 ns	0.6 *	0.61 *	0.87 **					
80dap	0.29 ns	0.51 ns	0.51 ns	0.85 **	0.97 *				
87dap	0.23 ns	0.4 ns	0.39 ns	0.72 *	0.89 **	0.96 ***			
94dap	0.14 ns	0.41 ns	0.31 ns	0.6 *	0.83 **	0.9 ***	0.96 ***		
AUDPC	0.35 ns	0.55 *	0.51 ns	0.83 *	0.96 ***	0.99 ***	0.97 ***	0.92 ***	
Yield	-0.22 ns	-0.47 ns	-0.3 ns	-0.67 *	-0.6 *	-0.71 ***	-0.64 *	-0.62 *	-0.68 *

Data significant at $\alpha < 0.05$ (*), $\alpha < 0.01$ (**), $\alpha < 0.001$ (***).

Table S1. Soil contents in two locations in Chimborazo province, Ecuador.

	Quimiag	Tunshi
Nitrogen (ppm)	84	47
hosphorus (ppm)	121	49
Sulfur (ppm)	0,79	0,78
Potassium (ppm)	6,4	11
Calcium (meq/100 ml)	14,1	8,8
Magnesium (meq/ml)	2,4	3,8
pH	5,52	6,31
Organic matter (%)	3,9	1,5

Chapter 8

General Discussion

Late blight disease of potatoes is a major constraint in the production of potatoes and is occurring globally as shown in Figure 1. It causes severe losses amounting to almost 1 billion euros in the European Union annually (Haverkort et al, 2008). Late blight disease increases the production costs because of fungicide applications with about 300 to 500 dollars per hectare in for instance the United States (Johnson et al, 2000; Guenther et al, 2001). In susceptible cultivars without fungicide use in Argentina a reduction of 35.6 % of the total yield was observed (Mantecon, 2009). In Ecuador, the pathogen may cause losses ranging from 28 to 100 % (Morales et al, 1995). The disease management costs may represent 20% of the total production costs in Ecuadorian conditions (Oyarzun, 2002).

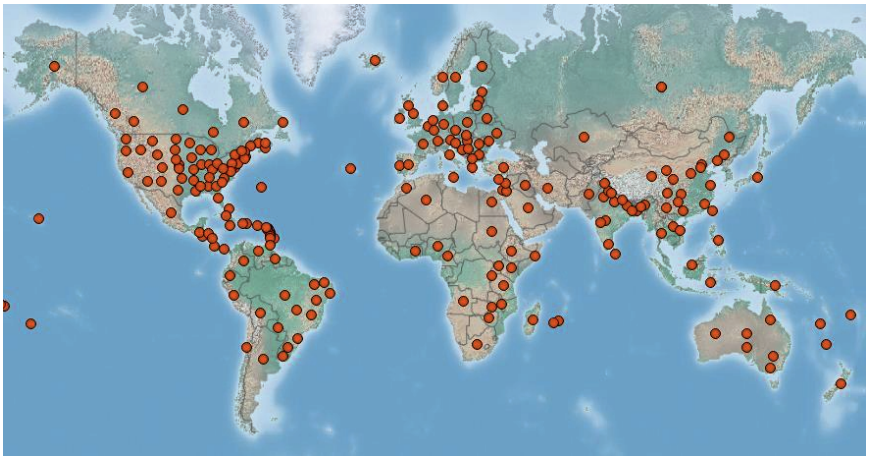


Figure 1. Global distribution map of *Phytopthora infestans*, the causal agent of late blight of potatoes (https://www.cabi.org/isc/datasheet/40970).

The main objective of this thesis was to improve the knowledge with regard to the variability of *Phytophthora infestans* populations in Ecuador and connected to that to identify potential resistance to late blight in the Ecuadorian potato landraces and some varieties. Attempts were also made to identify molecular markers associated to late blight resistance and to analyze the breeding work performed in Ecuador, particularly by INIAP, the National Agricultural Research Institute, which has been working on this topic for more than fifty years.

Late blight resistance

The efforts carried out in Ecuador over the last 60 years, with reference to potato breeding for late blight were reviewed in Chapter 2. Initially there were only isolated efforts evaluating

some landraces and varieties introduced in the early 1900´s. Later, some breeding activities were performed by botanists leading to a few released varieties from which a single variety, named Superchola still persists today because of its high demand in local markets (Gonzalez et al, 2017). Since its creation, the National Agricultural Research Institute of Ecuador (INIAP), has developed new varieties which were more productive and resistant to late blight. As a result, twenty one potatoes varieties have been released by INIAP.

According to Cuesta (2013) and Cuesta et al (2015), the potato ideotype in Ecuador required by farmers, traders and consumers varies a lot from one region to another, however sharing similar requirements, such as late blight resistance, high yield (>30 ton/ha), earliness (<150 days), shallow eyes, round shape, red, pink or yellow skin and yellow flesh tubers. Additionally they must have good culinary characteristics. Some of these traits, like tuber shape for example, are monogenic, but others like yield and late blight quantitative resistance are polygenic which would complicate the breeding process, making it difficult to develop new varieties (Cuesta, 2013).

Different breeding strategies have been used by INIAP, among them, clonal selection which rendered the first variety developed by INIAP (Santa Catalina). Another approach was the recurrent selection method that identified clones with resistance to the disease and using them as parents in order to obtain progenies with more resistance to *P. infestans*. Working in collaboration with the International Potato Center (CIP), advanced potato clones were introduced, characterized and selected, leading to the release of some varieties with monogenic resistance, which was effective at the beginning, but were later defeated (Revelo et al, 1997). An example, is the case of INIAP Cecilia and INIAP Gabriela varieties which lost their resistance to the disease shortly after their release (Forbes et al, 2012). This strategy was changed in order to select quantitative resistance to late blight, but rendering new varieties at that time, which were INIAP Fripapa, INIAP Rosita, INIAP Raymipapa and INIAP Victoria. Mutation breeding was also explored, with some results, but no improved varieties were developed (Lopez & Yanez, 2010). Introgression of genes from wild species was carried out crossing varieties with hybrids of *S. phureja* x *S. pausissectum*, so two varieties were obtained in this manner (INIAP-Natividad and INIAP-Estela).

In this thesis, as presented in Chapters 4 and 5, potato landraces were evaluated for their potential as a source for late blight resistance, particularly for quantitative resistance which is supposed to be more stable and durable. Few clones behaved with similar levels of resistance, like the improved varieties already available in the country. This agrees with several reports from Bolivia, Colombia, Ecuador, Chile and Perú, where most of the accessions of potato landraces evaluated were classified as moderately resistant to susceptible (Thurston et al, 1962, Cañizares & Forbes, 1995, Gabriel et al, 2007, Gabriel et al, 2013, Solano et al, 2014, Pérez et al, 2014). Since most of the accessions evaluated behaved as moderately resistant and susceptible, the survival of these landraces must be associated to some other traits.

Farmers may have conserved these landraces because of characteristics such as taste or culinary quality (Monteros-Altamirano, 2011). Cuesta et al (2012) identified landraces with

high contents of dry matter, polyphenols and carotenoids. Traits like frost and drought tolerance, and even medicinal uses were mentioned by farmers as a reason to keep planting potato landraces (Monteros-Altamirano, 2011). Other reasons for the conservation of these potatoes may be due to the cultivation practices performed by farmers as described by Monteros-Altamirano (2011), planting them in mixed plots which may reduce the severity of late blight, which agrees with what was observed by Andrivon et al (2003) and Pilet et al (2006). This practice, probably was not intentionally done for this purpose, however it allowed them to preserve those landraces susceptible to late blight up to now.

Those landraces that were as resistant as the improved varieties (described in Chapters 4 and 5) might be expected to perform fine since they were exposed to late blight disease in the field where several complex races are reported to occur naturally (Tello, 2008).

Pathogen variability

Additionally in this research, the pathogenic and the genetic variability of the *Phytophthora infestans* populations associated to the potato landraces in the provinces of Carchi, Chimborazo and Loja were studied (Chapter 3). Furthermore, we confirmed the prevalence of the clonal lineage EC-1 in the potatoes in Ecuador since the 1990s (Forbes et al, 1997).

Despite the fact that all the studied isolates in this research infecting Ecuadorian potato landraces belonged to the clonal lineage EC-1 exclusively, without sexual reproduction, it was observed that the population analysed was complex and virulent on 4 to 11 R-genes on potato differentials. Even more, genetic variability was identified among the clonal population as assessed with the aid of SSRs, meaning that despitethe lack of sexual recombination there are mechanisms of variation which contribute to the complexity of this population. Moreover, the occurrence of more than two alleles in the SSRs profiles was observed. We hypothesized that this maybe a mechanism for variability in a pathogen population which was supposed to be diploid and clonally reproduced, possibly by mutations, loss of chromosome regions or mitotic recombination. The occurrence of more than two alleles at a specific locus had been reported previously by several researchers (Knapova & Gisi, 2002; Lees et al, 2006; Chacón Acosta, 2007; Akino et al, 2009; Oliva Pérez, 2009; Cooke et al, 2012, Li et al, 2012 a,b). Recently, based on what we originally observed about the variability in number of alleles (Chapter 3, Delgado et al, 2013), it has been proposed that isolates belonging to clonal lineages like Blue_13, US-1 and EC-1, were actually triploid (Li et al, 2017). These triploid clonal lineages may shift to diploid stage when under stress conditions, thus this phenomena would contribute to the pathogen fitness by gene dosage or multiallelism, favouring its adaptation to environmental changes according to Li *et al* (2017).

The occurrence of particular SSR multilocus genotypes (MLG) of *Phytophthora infestans* that we found in this research (Chapter 3, Delgado et al, 2013), demonstrate that we can identify the predominant MLGs among the EC-1 clonal population, verify its movement, as well as their changes in abundance in different locations across the country and over the years. A

recent example of this, may be seen in a study were the *P. infestans* Indian population was characterized, identifying the MLG from different geographic areas, drawing conclusions about their origin and future actions to prevent the entry of new populations of the pathogen to that country (Dey et al, 2018). Other practical uses for this knowledge is that it opens the possibility of testing the late blight resistance of potatoes against specific MLGs which predominate in different areas in Ecuador or elsewhere.

Perspectives for late blight resistance breeding in Ecuador

The high diversity of the asexual clonal lineage of *P. infestans* in the country constitutes a challenge for potato breeding. Besides there are other traits that are needed for new varieties to be released in Ecuador. The consumer demands certain characteristics associated to the shape, skin, and flesh color, among others traits, making the development of new varieties and its adoption by farmers as well as by consumers more difficult.

The current breeding scheme followed by INIAP in Ecuador is presented in Figure 2. According to our findings in this research, to incorporate stable late blight resistance there are few resistant or tolerant potato accessions available, which could be used as progenitors (Chapters 4 and 5). Nevertheless, there are still accessions from the potato germplasm collection that must be characterized. Recently, Monteros-Altamirano et al (2017), using SSRs, reported that Ecuadorian landraces possess unique alleles and can be distinguished from European varieties, which means that they have variability still unexploited in breeding. For instance, some species like *S. phureja* and *S. andigena* are pointed out as sources of resistance in Bolivia, Colombia, Peru and Spain (Escallon et al, 2005; Gabriel et al, 2007; Gabriel et al, 2013; Pérez et al, 2014; Alor et al, 2015). One approach to improve the identification of sources of resistance to late blight may include the inoculation of detached leaves with the main dominant SSR multilocus genotypes of the different environments where we want to develop new varieties, preferable those predominant in the north, center and south of the country (Figure 3). This would aid to identify accessions with resistance to the local populations, taking into account that EC-1 has genetic variability (Chapter 3, Delgado et al, 2013). After this, the selected accession should be characterized for other valuable traits such as stress tolerance (drought and/or cold), nutritional or culinary properties, etc. This would be crucial, taking in account that despite, late blight being the main biotic stress affecting potatoes in Ecuador, the predominant variety planted in the country is the susceptible, Superchola (Buddenhagen et al, 2017, Gonzalez et al, 2017), so there are farmers and consumers demands related to quality traits that should be covered by the new potatoes varieties to be released. Of course these potatoes must then be tested in the field for their resistance and yield preferably in different locations as we have shown in Chapter 7, where variables like Relative Humidity or rainfall may change the status of varieties regarding late blight resistance. This was also observed in a group of clones and varieties when evaluated in three different provinces (Carchi, Pichincha and Chimborazo), with no differences or interaction for Iron and Zinc contents (Comina et al, 2017). In figure 4, a map of Ecuador is shown with the different conditions for rainfall and temperature in the Ecuadorian highlands, where the potatoes are predominantly cultivated.

In addition, as shown in Chapter 3, there will be genotypes within the EC-1 population that might be predominant in some sites. Adaptation trials must be carried out in more locations, as many as possible, in order to obtain more reliable conclusions. So 'hot spots´ for late blight epidemics must be identified across the highlands, which should be combined with favorable weather conditions for the disease development as well as a high genetic and pathogenic diversity of the pathogen, such as, in the case of rice and the blast caused by *Magnaphorthe oryzae* (Correa-Victoria & Zeigler, 1993) allowing to identify varieties with stable resistance. In figure 5, we propose including a screening of potatoes or varieties for late blight resistance against the main MLG across the country, using disease hot spots for identifying highly resistant potatoes to the disease.

Introduction of resistance genes is also a must in order to have alternatives to manage the pathogen that we found to have diversity despite the lack of sexual recombination. Another alternative to be explored is the use of the germplasm collection´s from INIAP, which has been preserved as seeds of *Solanum* accessions from different origins that should be characterized in order to find if there is any valuable source for late blight resistance (Tapia et al, 2008). *Solanum* accessions different from *S. demissum* must be used in the breeding process, but it is necessary to know if these Solanums are effective against Ecuadorian populations of *P. infestans*. An example of this can be evidenced in the case of *Rpi-vnt1.1*, a broad-spectrum resistance gene. Pel (2010) concluded that it was ineffective against EC-1, but Roman et al (2017) found that this gene was able to resist infection from variants of EC-1 which had a low expression of the *Avr-vnt1.1* gene, so the effectiveness of a R gene must necessary be tested against the predominant MLG of the pathogen population.

Alternatives for speeding up the breeding process of late blight resistant varieties must be considered, as was mentioned before, but the development of transgenic or cysgenic varieties is not possible since there is a legal ban (Article 401 of the Ecuadorian Constitution), on the use of these GM improved varieties. More recently, it has been suggested that the gene editing technology which leads to targeted changes in the plant genome without introducing alien DNA (Wiel et al, 2017), may not be limited due to the actual legal restrictions over the GMOs according to Vincelli (2016). So the introduction in the country of varieties or clones resistant to late blight developed with this technology seems to be possible. Recently, a judgment from the European Union, has decided that genetic modified plants with this technology must be regulated following the existing GMO rules in the EU, making this alternative highly unlikely.

In Chapter 6, we attempted to map markers associated to late blight resistance, with no success. Nevertheless, the use of marker assisted selection is possible since it allows to identify known late blight resistance genes in breeding populations (Sliwka et al, 2010) and in a germplasm collection by association mapping (Alvarez et al, 2017). The fact that these markers were not identified for the more quantitative related field resistance might be due to the lack of polymorphism between the used populations or the fact that these field resistances are too low in resistance level to differentiate them from susceptible genotypes. This then brings up the question whether novel major R genes in stacked form should be introduced into Ecuadorian genotypes. There are currently quite a number of cloned R genes available

including markers and effectors and they could be used to obtain new varieties with pyramided R genes (Haverkort et al, 2016).

One crucial step for potato production in Ecuador is to change the use of tubers from previous harvest as seeds, which includes 85% of the farmers (Monteros-Guerrero, 2016). This situation leads to poor seed quality which affects the yields (Navarrete et al, 2017). As an alternative, the use of improved varieties must be promoted by financial or technical assistance provided by governmental specialized offices, thus leading to the adoption of new varieties, allowing to increase the national yields and incomes of farmers.

In conclusion, potato late blight breeding is still a major challenge for Ecuadorian research and researchers. Opportunities to speed up the process to obtain resistant varieties, like marker assisted selection must be explored. Improving the procedures for more accurate characterization with the aim of identifying varieties with broad spectrum resistance may be achieved by implementing the screening with the predominant MLGs. Modern techniques, such as transgenesis, cisgenesis, or gene editing, may not be feasible in the short term since the legal framework in Ecuador does not allow the use of them.

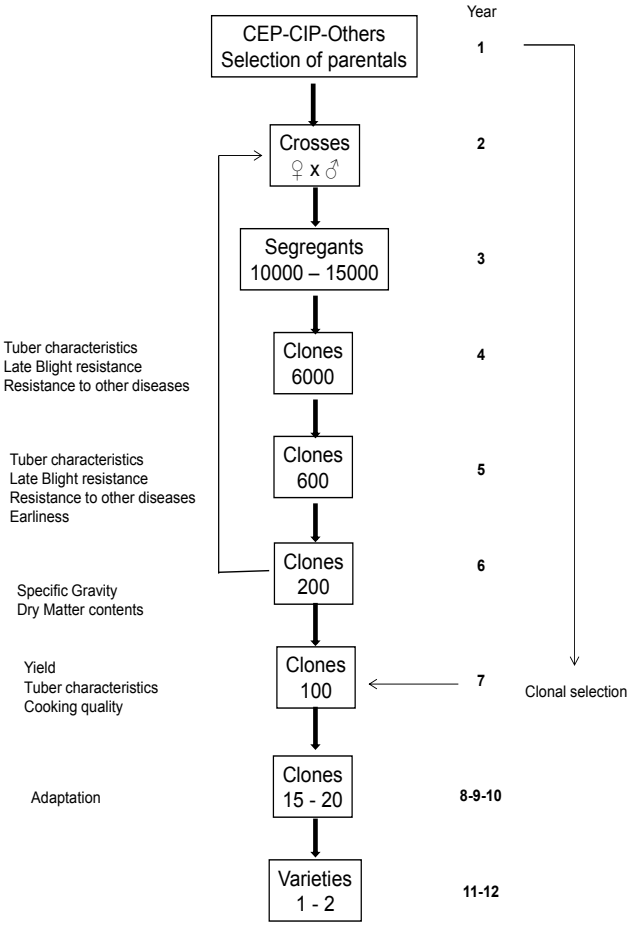


Figure 2. Breeding processes carried out by the Potato Program of INIAP (Adapted from Cuesta, 2011, Cuesta et al, 2015).

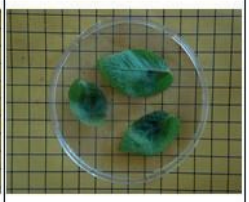
Accessions	Multilocus Genotypes		
	North	Center	South
1 . . . n			
Resistant check			
Susceptible check			

Figure 3. Screening of potato varieties, introduced potatoes and wild relatives against predominant *P. infestans* Multilocus Genotypes from North, Center and South areas of Ecuador.

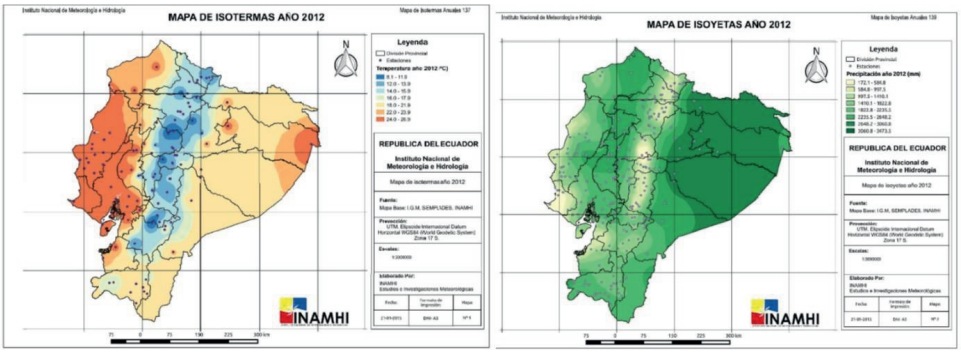


Figure 4. Maps of Isotherms (left) and Isoyets (right) of Ecuador in 2012. (INAMHI, 2015).

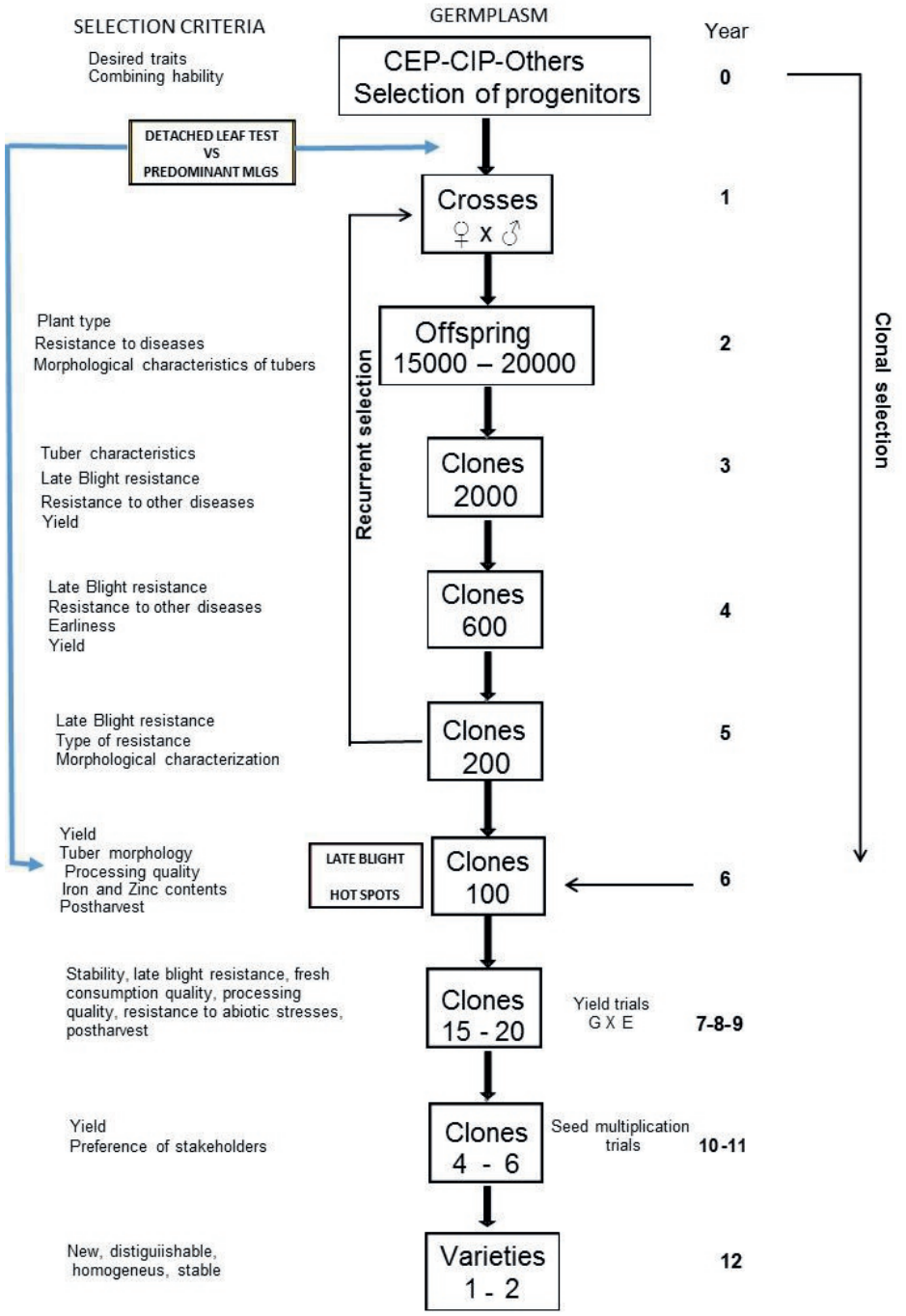


Figure 5. Breeding process for late blight resistance including detached leaf test against main Multilocus Genotypes of *P. infestans* and the use of hot spots field locations in order to improve for high resistance against late blight.

Literature cited

Akino S, Baba Y, Kondo N, 2009. Use of microsatellite marker Pi26 for rapid discrimination of five Japanese genotypes of *Phytophthora infestans*. *Journal of General Plant Pathology* 75, 349-352.

Alor N, López-Pardo R, Barandalla L, Ríos D, Ritter E, Ruíz de Galarreta JI. 2015. New sources of resistance to potato pathogens in old varieties of the Canary Islands. Potato Research 58, 135-146.

Álvarez, M. F., Angarita, M., Delgado, M. C., García, C., Jiménez-Gomez, J., Gebhardt, C., Mosquera, T. 2017. Identification of Novel Associations of Candidate Genes with Resistance to Late Blight in Solanum tuberosum Group Phureja. Frontiers in Plant Science, 8, 1040. http://doi.org/10.3389/fpls.2017.01040

Andrivon D., Lucas J.M., Ellissèche D. 2003. Development of natural late blight epidemics in pure and mixed plots of potato cultivars with different levels of partial resistance. Plant Pathology 52: 586-594.

Buddenhagen, C.E., Hernandez, J.F., Andersen, K.F., Andrade-Piedra, J., Forbes, G.A., Kromann, P., Thomas-Sharma, S., Useche, P., Garrett, K.A. 2017. Epidemic Network Analysis for Mitigation of Invasive Pathogens in Seed Systems: Potato in Ecuador. Phytopathology 107(10):1209-1218. doi: 10.1094/PHYTO-03-17-0108-FI.

Cañizares G., Forbes, G. 1995. Foliage resistance to Phytophthora infestans (Mont.) de Bary in the Ecuadorian national collection of Solanum phureja ssp. phureja Juz. & Buk. Potato Research 38: 3-10.

Chacón MG, 2007. Variability of Phytophthora infestans sensu lato in the highland tropics of Ecuador. Zurich: Switzerland: Swiss Federal Institute of Technology Zurich, PhD. Thesis.

Comina, P., Rivadeneira JE., Cuesta, HX. 2017. Estudio de la interacción genotipo por ambiente en papa para resistencia a tizón tardío y contenidos de Fe y Zn. In Kromann P, Cuesta X, Montero BR, Cuasapaz P, León-Reyes A, Chulde A. Memorias del VII Congreso Ecuatoriano de la Papa. Junio, 29 - 30 de 2017. Tulcán, Carchi, Ecuador. Pp. 39-40.

Correa-Victoria FJ, Zeigler RS. 1993. Pathogenic variability in Pyricularia grisea at a rice blast ´hot spot´ breeding site in Eastern Colombia. Plant Disease 77: 1029-1035.

Cuesta, X. 2011. Recursos genéticos de la papa y fitomejoramiento. In I Expo Congreso Nacional de Papa (Expopapa). 25-27 de noviembre de 2010 Ibarra, Ecuador.

Cuesta, X. 2013. Potato quality traits: variation and genetics in Ecuadorian potato landraces. PhD. Thesis. Wageningen, The Netherlands, Wageningen University.

Cuesta-Subia, X., Monteros-Altamirano, A. van den Berg, R., Visser, R.G.F., Vosman, B. 2012. Tuber quality characteristics of Ecuadorian potato landraces and farmer preferences. Revista Latinoamericana de la Papa 17(1): 125-151.

Cuesta, X., Rivadeneira, J., Monteros, C. 2015. Mejoramiento genético de papa: Conceptos, procedimentos, metodologías y protocolos. Quito, Ecuaodr: Instituto Nacional de Investigaciones Agropecuarias. 62 p.

Delgado, R. A., Monteros-Altamirano, A. R., Li, Y., Visser, R. G. F., van der Lee, T. A. J., Vosman, B. 2013. Large subclonal variation in Phytophthora infestans populations associated with Ecuadorian potato landraces. Plant Pathology, 62: 1081–1088. doi: 10.1111/ppa.12039

Dey T, Saville A, Myers K, Tewari S, Cooke DEL, Tripathy S, Fry WE, Ristaino JB, Guha Roy S. 2018. Large sub-clonal variation in Phytophthora infestans from recent severe late blight epidemics in India, Scientific Reports 8:4429 | DOI:10.1038/s41598-018-22192-1

Escallon, R., Ramírez, M., Ñústez, C.E. 2005. Evaluación del potencial de rendimiento y de la resistencia a Phytophthora infestans (Mont. de Bary) en la colección de papas redondas amarillas de la especie Solanum phureja (Juz. et Buk.). Agronomía Colombiana 23 (1): 35-41.

Forbes GA. 2012. Using Host Resistance to Manage Potato Late Blight with Particular Reference to Developing Countries. Potato Research (2012) 55:205–216 DOI 10.1007/s11540-012-9222-9

Gabriel J, Plata G, Cadima X, Franco J. 2013. Solanum phureja Juz et Burk.: Valuable source of genetic resistance to potato late blight (Phytophthora infestans (Mont.) de Bary). Revista Latinoamericana de la Papa 17 (2), 131-142.

Guenthner, J.F., Michael, K.C., Nolte, P. 2001. The economic impact of potato late blight on US growers. Potato Research 44: 121. https://doi.org/10.1007/BF02410098

Gonzalez S, Salazar C, Monteros A. 2017. Rendimientos de papa (Solanum tuberosum L.) en el Ecuador: Ciclo 2016. In Kromann P, Cuesta X, Montero BR, Cuasapaz P, León-Reyes A, Chulde A. Memorias del VII Congreso Ecuatoriano de la Papa. Junio, 29 - 30 de 2017. Tulcán, Carchi, Ecuador. Pp. 15-16.

Haverkort AJ, PM Boonekamp, R Hutten, E Jacobsen, LAP Lotz, GJT Kessel, JH Vossen & RGF Visser (2016). Durable late blight resistance in potato through dynamic varieties obtained by cisgenesis: scientific and societal advances in the DuRPh project. Potato Research 59:35-66.

INSTITUTO NACIONAL DE METEOROLOGÍA E HIDROLOGÍA – INAMHI. 2015. Anuaria meteorológico 2012. Quito, Ecuador; INAMHI.

Johnson, D. A., Cummings, T. F., and Hamm, P. B. 2000. Cost of fungicides used to manage potato late blight in the Columbia Basin: 1996 to 1998. Plant Disease 84:399-402.

Knapova G, Gisi U, 2002. Phenotypic and genotypic structure of Phytophthora infestans populations on potato and tomato in France and Switzerland. Plant Pathology 51, 641–53.

Lees AK, Wattier R, Shaw DS, Sullivan L, Williams, NA, Cooke DE, 2006. Novel microsatellite markers for the analysis of Phytophthora infestans populations. Plant Pathology 55, 311-319.

Li Y, van der Lee T, Evenhuis A et al., 2012a. Population dynamics of Phytophthora infestans in the Netherlands reveals expansion and spread of dominant clonal lineages and virulence in sexual offspring. Genes, Genomes, Genetics 2, 1529–40.

Li Y, van derLee T, Zhu JH et al., 2012b. Population structure of Phytophthora infestans in China – geographic clusters and presence of the EU genotype Blue_13. Plant Pathology. Doi: 10.1111/j.1365-3059. 2012.02687.

Li, Y., Shen, H., Zhou, Q., Qian K., Lee T., Huang. S. 2017. Changing ploidy as a strategy: the Irish potato Famine pathogen shifts ploidy in relation to its sexuality. Molecular Plant Microbe Interactions 30 (1), 45-52. http://dx.doi.org/10.1094/MPMI-08-16-0156-R

Mantecon JD. 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.

Monteros-Altamirano, A. 2011. Potato landraces: description and dynamics in three areas of Ecuador. PhD. Thesis. Wageningen, The Netherlands, Wageningen University.

Monteros-Altamirano, A., Buitron-Bustamante, J., Orbe-Vergara, K., Cuesta-Subia, X. 2017. Ecuadorian potato landraces: traditional names and genetic identity. Revista Fitotecnia Mexicana 40(4), 481-289.

Monteros-Guerrero, A. 2016. Rendimientos de papa en el Ecuador. Primer ciclo 2016. Quito: Ministerio de Agricultura, Ganadería y Pesca. 10 p. http://sipa.agricultura.gob.ec/descargas/estudios/rendimientos/papa/rendimiento_papa_2016.pdf

Morales, R.; Revelo, J.; Suárez, G.; Forbes, G. Muñoz, F. 1995. Relación entre el ataque de Phytophthora infestans y la producción en el cultivo de papa (Solanum tuberosum). In Memorias XVII Reunión de la Asociación Latinoamericana de la Papa (ALAP). Mérida, Venezuela. Pp. 73-74.

Navarrete I, Panchi N, Andrade-Piedra JL. 2017. Potato crop health quality and yield losses in Ecuador. Revista Latinoamericana de la Papa 21 (2): 69 – 88.

Revelo, J., Garcés, S., Andrade, J. 1997. Resistance of commercial potato varieties to the attack by Phytophthora infestans in Ecuador. CIP Circular: 20-21.

Sliwka, J., Jakuczun, H., Kamiński, P., Zimnoch-Guzowska, E. 2010. Marker-assisted selection of diploid and tetraploid potatoes carrying Rpi-phu1, a major gene for resistance to Phytophthora infestans. Journal of Applied Genetics 51(2), 133–140.

Solano J, Acuña I, Esnault F, Brabant P. 2014. Resistance to Phytpthora infestans in Solanum tuberosum landraces in Southern Chila. Tropical Plant Pathology 39 (4), 307-315.

Oliva RF. 2009. Occurrence of sympatric Phytophthora species in the highland of Ecuador. Zurich, Switzerland: Swiss Federal Institute of Technology Zurich, PhD. Thesis.

Oyarzún P, Gallegos P, Asaquibay C, Forbes G, Ochoa J, Paucar B, Prado M, Revelo J, Sherwood S, Yumisaca F. 2002. Manejo integrado de plagas y enfermedades. In: Pumisacho M, Sherwood S, eds. El Cultivo de la Papa en Ecuador. Quito, Ecuador: INIAP/CIP, 85–169.

Pérez W, Ñahui M, Ellis D, Forbes GA. 2014. Wide phenotypic diversity for resistance to Phytophthora infestans foundin potato landraces from Perú. Plant Disease 98, 1530-1533.

Pilet F., Chacón G., Forbes G.A., Andrivon D. 2006. Protection of Susceptible Potato Cultivars Against Late Blight in Mixtures Increases with Decreasing Disease Pressure. Phytopathology 96 (7): 777-783.

Roman, M.L., Izarra, M., Lindqvist-Kreuze, H., Rivera C, Gamboa S, Tovar JS, Forbes GA, Kreuze JF, Ghislain M. 2017. R/Avr gene expression study of Rpi-vnt1.1 transgenic potato resistant to the Phytophthora infestans clonal lineage EC-1 . Plant Cell Tiss Organ Cult 131: 259-268. https://doi.org/10.1007/s11240-017-1281-9

Tapia C, Zambrano E, Monteros A. 2008. Estado de los recursos fitogenéticos para la agricultura y la alimentación en Ecuador: A mas uso, mas conservación. Quito, Ecuador: INIAP, Estación Experimental Santa Catalina, Departamento Nacional de Recursos Fitogenéticos. Publicación Miscelánea no. 144.

Tian Y, Sun J, Li H, Wang G, Ma D, Liu D, Quan J, Shan W. 2015. Dominance of a single clonal lineage in the Phytophthora infestans population from northern Shaanxi, China revealed by genetic and phenotypic diversity analysis. Plant Pathology (2015) 64, 200–206. Doi: 10.1111/ppa.12251

Vincelli, P. 2016. Genetic Engineering and Sustainable Crop Disease Management: Opportunities for Case-by-Case Decision-Making. Sustainability 8, 495. https://doi.org/10.3390/su8050495

Wiel, C.C.M., Schaart, J.G., Lotz, L.A.P., Smulders, J.M.J. 2017. New traits in crops produced by genome editing techniques based on deletions. Plant Biotechnology Reports11(1): 1–8. doi: 10.1007/s11816-017-0425-z

Summary

Potato is one of the most important food crops in Ecuador. It is produced mostly by small scale farmers. Late blight is the main disease that affects potatoes in the country, which reduces the yields. From its early beginnings right until now, late blight resistance research and breeding remains the most important Ecuadorian research goal in potato breeding.

In Chapter 2, the potato breeding history in the country and the main results achieved in the last 60 years are reviewed. Although progress has been made over these years durable late blight resistant varieties have not yet been created. Additionally, discussed is the possible reason for a poor adoption of late blight resistant improved varieties. Farmer preferences as well traders and consumers have their role in the adoption of varieties. So there is a need for a formal seed system as well as incentives to lead farmers to adopt new varieties.

The pathogenic and genetic variability of the *Phytophthora infestans* populations associated to potato landraces in three provinces (Carchi, Chimborazo and Loja) of Ecuador was analysed in Chapter 3. Despite the fact that all the isolates were of the A1 mating type, which implies that no sexual recombination occurs, the population analysed was complex and virulent on 4 to 11 R-genes. From the 66 isolates studied, 39 races and 31 multilocus genotypes were identified. All the isolates belonged to the clonal lineage EC-1 which predominates in Ecuador since the 1990s. The analysis performed using a set of 12 SSR markers lead to identify variability within a clonal population. This opens the possibility to monitor the predominant genotypes of the pathogen.The results suggest high mutation rates and little or no selection for specific genotypes.

Then inn Chapter 4, a selection of potato landraces was evaluated for its potential as a source for late blight resistance. Two field trials were conducted in San Pedro de Huaca, Carchi province. Additionally, an aggressiveness test was conducted in the laboratory with three *Phytophthora infestans* isolates which were inoculated on detached leaflets of a set of five selected landraces and two commercial varieties. In the field trials, the landraces Santa Rosa Amarilla, Uva, Coneja Blanca and Botella were partially tolerant to late blight and grouped with INIAP Estela, Fripapa and Natividad varieties. In the aggressiveness test, it was observed that the ranking of the genotypes varied depending on the isolate. The resistance detected seems to be of a similar level as that of improved varieties released in the country. Detached leaf assays may give an indication on the reaction of the genotypes, but field evaluation is necessary to confirm the trait. For future breeding, the resistant accessions observed can be used in combination with other quantitative resistant genotypes and/or carriers of major resistance genes to breed for (quantitative) resistant varieties.

A field experiment in Chapter 5 was carried out to assess resistance or susceptibility to late blight of 31 Ecuadorian potato landraces from a new germplasm collection obtained in Carchi, Chimborazo and Loja which were genetically different. The landraces under study showed different responses to late blight in the experimental field. Few landraces demostrated field resistance similar to the improved variety INIAP-Fripapa. For breeding purposes we can search among the collection of potato landraces for late blight resistance, but few accessions with field resistance may be expected. The introduction of new sources of resistance which

can be cross with local germplasm may be an alternative. Additionally, a survey among 145 farmers growing potato landraces in these three provinces identified late blight as the main disease affecting their potatoes.

The breeding process for obtaining new late blight resistant potatoes takes several years. Potatoes landraces are under study as a source of late blight resistance. One of the species present in Ecuador is *Solanum phureja*. This species had been reported to present quantitative resistance to the pathogen. Some previous research by several authors have identified Quantitative Trait Loci (QTLs) associated for resistance to the disease in *S. phureja*. The identification of QTLs associated to the desired trait may be use as a tool to speed up the breeding process. In Chapter 6, an attempt to map resistance to late blight was made using a segregating population of a *Solanum phureja* cross from the Ecuadorian Potato Collection. DArT markers were utilized for genotyping. No QTLs were identified, probably due to lack of a sufficient contrasting resistance level in the studied offspring plants. Further studies will be necessary to confirm this.

The late blight susceptibility and yield of fifteen potato varieties was evaluated in two locations in Ecuador, in Chimborazo province in Chapter 7. There were significant differences observed for environments, genotypes and the interaction between these two sites. For one of this sites, Tunshi, there was no correlation among yield and late blight assessments and AUDPC. Meanwhile, in Quimiag, the opposite situation was observed. Carolina and 99_66_6, were the best performing clones with regard to late blight resistance. Carolina, INIAP-Estela and INIAP-Natividad had the best yields in both places. The variability observed shows the difficulties for breeding potatoes in Ecuador. Nevertheless, the varieties that performed good in both places are valuable for breeding and should be used as parental clones in the country.

Finally, in Chapter 8, the main findings are summarized and discussed for its use in future work with late blight in the country. It is discussed why despite the breeding efforts performed in the country, there is still low adoption of the improved late blight resistance varieties and why a susceptible one persist. Also discussed is the value of potato landraces as a source of late blight resistance for breeding purposes. The role of the variability of the clonal pathogen population is discussed. The monitoring of the main multilocus genotypes and screening the germplasm against them must be routine in the country. Introduction of new and effective late blight resistance genes is a need in order to obtain better varieties for this trait. Finally, the alternatives for improving the breeding process for late blight resistance are expressed in regards to the present legal frame in Ecuador which despite the limitations still requires change for improvement.

Resumen

La papa es uno de los más importantes cultivos del Ecuador. Este es cultivado por pequeños agricultores principalmente. El tizón tardío es la principal enfermedad que afecta al cultivo en el país, la cual reduce rendimientos. Así como en los inicios hasta la actualidad, la investigación y mejoramiento para resistencia a tizon tardío continúa siendo el objetivo principal en el mejoramiento del cultivo de la papa.

En el Capítulo 2, la historia del mejoramiento del cultivo de la papa en el país es revisada, así como los principales logros obtenidos en los últimos 60 años. A pesar del progreso realizado a través de estos años, resistancia durable a tizon tadio aun no ha sido desarrollada. Además, se discute los posibles motivos para la pobre adopción de variedades de papa mejoradas para resistencia al tizón tardío. Las preferencias de los agricultores, así como la de los comercializadores y consumidores jugarían un rol en la adopción de las variedades. Existe además la necesidad de un sistema formal de producción de semillas de papa, así como de incentivos para motivar la adopción de nuevas variedades.

La diversidad genética y patogénica de las poblaciones de *Phytophthora infestans* asociadas a papas nativas en tres porvincias (Carchi, Chimborazo y Loja) de Ecuador fue analizada, en el capítulo 3. A pesar del hecho de que todos los aislamientos correspondieron al tipo de apareamiento A1, por lo que no habría recombinación sexual, la población estudiada fue compleja y virulenta para 4 hasta 11 R-genes. De los 66 aislamientos estudiados, 39 razas y 31 genotipos multilocus fueron identificadas. Todos los aislamientos pertenecieron al linaje clonal EC-1, el cual predomina en Ecuador desde los noventas. Los análisis realizados utilizando un set de 12 marcadores SSR abren la posibilidad de monitorear los genotipos predominantes en la población del patógeno. Los resultados sugieren altas tasas de mutacion y poca o ninguna selección para genotipos específicos.

En el Capítulo 4, un grupo seleccionado de papas nativas fue evaluado por su potencial como fuentes de resistencia al tizón tardío. Dos ensayos a campo fueron conducidos en San Pedro de Huaca, provincia del Carchi. Adicionalmente, una prueba de agresividad fue llevada a cabo en laboratorio con tres aislamientos de *Phytophthora infestans*, los cuales fueron inoculados en folíolos desprendidos de un grupo de cinco papas nativas y dos variedades comerciales. En los ensayos a campo, las variedades nativas Santa Rosa Amarilla, Uva, Coneja Blanca y Botella fueron parcialmente tolerantes al tizón tardío y se agruparon junto con las variedades mejoradas INIAP Estela, Fripapa y Natividad. En la prueba de agresividad, se observó que el ranking de los genotipos variaba dependiendo del aislamiento utilizado. La resistencia detectada parece ser de nivel similar al observado en las variedades mejoradas liberadas en el país. Las pruebas utilizando folíolos desprendidos pueden dar indicios sobre la reacción de los genotipos, pero los ensayos a campo son necesarios para confirmar el carácter. Para futuros trabajos en mejoramiento, las accesiones resistentes o tolerantes observadas pueden ser utilizadas en combinación con otros genotipos con resistencia cuantitativa y/o portadores de genes mayores de resistencia para mejorar variedades con resistencia cuantitativa ya existentes.

Un experimento a campo en Capítulo 5, fue llevado a cabo con la finalidad de evaluar la resistencia o susceptibilidad de 31 papas nativas ecuatorianas pertenecientes a nueva colección de germoplasma obtenida en Carchi, Chimborazo y Loja, las cuales eran genéticamente diferentes. Las papas nativas estudiadas en este trabajo tuvieron diferentes

respuestas frente al tizon tardío en campo. Pocas de estas papas nativas mostraron alto nivel de resistencia en campo similar al de la variedad INIAP-Fripapa. Para fines de mejoramiento, nosotros podemos explorar la colección de papas nativas en busca de resistencia al tizón tardío, pero pocas accesiones con resistencia en campo serían de esperarse. La introducción de nuevas fuentes de resistencia que puedan ser cruzadas con germoplasma local puede ser una alternativa. Adicionalmente, en una encuesta realizada entre 145 productores de papa en estas tres provincias, estos identificaron al tizón tardío como la principal enfermedad afectados sus cultivos.

El proceso de mejoramiento genético para la obtención e nuevas variedades de papa toma muchos años. Las papas nativas están bajo studio como una possible Fuente de Resistencia al tizón tardío. Una de las especies presentes en el país es *Solanum phureja*. Esta especie ha sido reportada como portadora de resistencia cuantitativa al patógeno. Investigaciones realizadas previamente por otros investigadores han identificado la ocurrencia de QTLs asociados a resistencia a la enfermedad en *S. phureja*. La identificación de QTLs asociados a este carácter podría ser una herramienta para acelerar el proceso de mejoramiento. En el Capítulo 6, un tentativa de mapear la resistencia al tizon tardío fue llevada a cabo utilizando un población segregante de *Solanum phureja* pertenecientes a la Colección Ecuatoriana de Papa (CEP). Marcadores DarT fueron utilizados para el genotipado. No fueron identificados QTLs, probablemente debido a la falta de un contraste suficiente en el nivel de resistencia en las plantas de la progenie estudiada. Más estudios serán necesarios para confirmar esto.

La susceptibilidad al tizón tardío y rendimiento de quince variedades de papa fue evaluada en dos localidades en la provincia del Chimborazo, en el Capítulo 7. Diferencias significativas para ambientes, genotipos y la interación entre ambos fueron observadas. En una de las localidades, Tunshi, no se observó correlación entre rendimiento, mediciones de severidad de tizón tardío y Área Bajo la Curva de Progreso de la Enfermedad (ABCPE). En tanto que, en Quimiag, la situación opuesta fue observada. Carolina y 99_66_6, tuvieron la mejor performance en cuanto a resistencia al tizón tardío. Carolina, INIAP-Estela e INIAP-Natividad presentaron los mejores rendimientos en ambos sitios. La variabilidad observada pone en evidencia las dificultades que existen en el mejoramiento de papa en Ecuador. Sin embargo, las variedades que tuvieron buen comportamiento en ambas localidades tendrían valor para mejoramiento genético y deberían utlizarse como líneas parentales en el país.

Finalmente, en el Capítulo 8, los principales hallazgos son resumidos y discutidos para su uso en el futuro trabajo con el tizón tardío en el país. Se discute por qué a pesar de los esfuerzos realizados en mejoramiento genético en papa en el país, aún hay baja adopción de variedades mejoradas con resistencia al tizón tardío y por qué una variedad susceptible persiste. Además es discutido el valor de las papas nativas como fuente de resistencia al tizón tardío para fines de mejoramiento. El rol de la variabilidad de la población clonal del patógeno es discutido. El monitoreo de los principales genotipos multilocus y la evaluación del germoplasma contra ellos debería ser una rutina en el país. La introducción de nuevos y efectivos genes de resistencia contra el tizón tardío es una necesidad con la finalidad de obtener variedades mejoradas por ese carácter. Por último, alternativas para acelerar el proceso de mejoramiento por resistencia al tizón tardío son presentadas teniendo en cuenta el marco legal vigente en Ecuador, el cual a pesar de las limitaciones permite posibilidades de perfeccionamiento.

Acknowledgements

I would like to thank my promotor Dr. Richard Visser for been a guide and for his unconditional support during all the development of my PhD. Thank you for not given up. Also, I would like to express my gratitude to Dr. Daniel Danial for his advice during all this time. I want to thank Dr. Erwin van der Vossen and Dr. Ben Vosman, both supervisors in different periods of my PhD for their assistance. Also, to Theo van der Lee, Ying Lee and Jack Vossen for their participation. I would also like to thank all researchers and colleagues in Wageningen University for their comments and assistance, among them Dirk (+), Dirkjan, Koen and some more. To all secretariat staff for their kind assistance every time when I needed.

My appreciation to my fellow PhD colleagues Alvaro Monteros and Xavier Cuesta with whom I started this journey.

Special mention to Herma Koehorst-van Putten for your kind way of been, not just for me also for my wife and daughter. To Animesh Acharjee and Luis Montes for your friendship and mutual support. To my officemates Sammeer, Eric, Diana and Dennis. To my fellow collegues Anitha, Shital, Ningwen, Nicolas, Arwa, Leila, Paula and many others.

To the group of the Catholic choir and the Latin American community in Wageningen, Luis, Katarina, Caucasella, Rosa Elena, Guillermo, and many others.

To my former colleagues of the Potato Research Program of INIAP, Iván, Cecilia, José, Elizabeth, Efren and so more for their assistance in the PhD research and for made me part of the team.

To the staff of INIAP`s offices in Carchi, Chimborazo and Austro Experimental Station for their assistance when needed.

Finally, I would like to express my gratitude to my parents Julio César and Pilar, for their faith and love. I should mention that my father is the role model of what I ever wanted to be as an agricultural scientist, you deserve more recognition for your great career. And for whom are the light in life, my wife Jennifer and my daughter Silvia, thank you for your love and support even in my darkest moments and for your sacrifice during these years, I would need an extra life to compensate both of you for been there with me.

About the author

Ricardo Delgado was born on the 22th of February 1972. He obtained a degree as an Agricultural Engineer in 1997 at the Universidad de la República, Uruguay. Later, he earned a MSc degree in Plant Pathology at the Universidade de Passo Fundo, Brasil in 2001. That same year, he joined the National Agricultural Research Institute (Instituto Nacional de Investigaciones Agropecuarias – INIAP) in Ecuador. The first years he worked in the Tropical Agricultural Research Station 'Pichilingue' mainly with cocoa diseases. After that he continued researching in banana and rice diseases in the Agricultural Experimental Station 'Boliche'. Additionally, he was a Plant Pathology guest lecturer in the Escuela Superior Politécnica Agropecuaria de Manabí (ESPAM) from 2005-2006. In 2007, he started his PhD thesis on late blight, studying the pathogen variability and disease resistance on potato in the Laboratory of Plant Breeding, Wageningen University and Research Center (WUR). This same year he joined the Potato Research Program in INIAP located in the Agricultural Research Station 'Santa Catalina' where he worked with late blight disease on potatoes. Lately, he has been in charge of the banana and plantain research in the Agricultural Research Station 'Litoral Sur' dealing with red rust thrips, Moko disease, soil health, among other topics. Additionally, has acted as Scientific Advisor of the Fusarium Tropical Race 4 Wilt of Musaceae Prevention Committee in Ecuador.

List of publications

DELGADO, R. & VOSSEN, VAN DER, E. 2008. Selección de parentales resistentes al tizón tardío en especies diploides de *Solanum* spp.. Summary. *In* III Congreso Nacional de la Papa. Junio 18 – 20, 2008. Quito, Ecuador. CD-ROM.

DELGADO, R. & VOSSEN, VAN DER, E. 2008. Evaluación de resistencia al tizón tardío en introducciones diploides de *Solanum* spp.. Summary. *In* Memorias XXIII Congreso Latinoamericano de la Asociación Latinoamericana de la Papa/VI Seminario Latinoamericano de Uso y Comercialización de la Papa. 30 de noviembre al 6 de diciembre de 2008. Mar del Plata, Universidad Nacional de Mar del Plata. Pp. 191-192.

DELGADO, R.A. & VOSMAN, B. 2009. Ecuadorian native potatoes a source for late blight resistance. Tropical Plant Pathology 34 (Suppl.): 245. Presented in XLII Congresso Brasileiro de Fitopatologia. Rio de Janeiro, 3 - 7 de agosto, 2009.

DELGADO, R.A. & VOSMAN, B. 2010. Caracterización de razas de *Phytophthora infestans* asociadas a papas nativas en la provincia del Carchi, Ecuador. In I Congreso Internacional de Investigación y Desarrollo de Papas Nativas. Quito, Ecuador.

DELGADO, R.A. & VOSMAN, B. 2010. Frecuencia de factores de virulencia de *Phytophthora infestans* en papas nativas en Carchi, Ecuador. In Memoria XXIV Congreso de la Asociación Latinoamericana de la Papa. Cusco, Perú. Pp. 261-262.

CUESTA X., RIVADENEIRA J., YANEZ., DELGADO R., TELLO C., RIERA W., HINOJOSA L., CARRERA E., REINOSO I. 2010. Caracterización de papas nativas Ecuatorianas para resistencia a factores bióticos, abióticos y calidad In: Memorias I Congreso Internacional de papas nativas. Quito-Ecuador. p 34-37

DELGADO, R.A.; MONTEROS-ALTAMIRANO, A.R.; LI, Y.; VISSER, R.G.F.; LEE T.A.J., VOSMAN, B. 2013. Large subclonal variation in *Phytophthora infestans* populations associated with Ecuadorian potato landraces. Plant Pathology 62: 1081–1088. Doi: 10.1111/ppa.12039.

MONTEROS-ALTAMIRANO, A., CUESTA, X., DELGADO, R., VAN DEN BERG, R., VISSER, R., VOSMAN, B. 2011. Papas nativas: conservación y diversidad genética en tres áreas de Ecuador. Memorias VIII Simposio Internacional de Recursos Genéticos de América Latina y el Caribe – SIRGEALC. November 21-23, 2011. Quito, Ecuador. Pp. 211-213.

Education Statement of the Graduate School

Experimental Plant Sciences

Issued to: **Ricardo Delgado**
Date: **21 October 2019**
Group: **Laboratory of Plant Breeding**
University: **Wageningen University & Research**

1) Start-Up Phase	date	cp
► **First presentation of your project**		
Genetic dissection of late blight resistance in cultivated diploid Solanum species and modern potato varieties of Ecuador	01 Nov 2007	1.5
► **Writing or rewriting a project proposal**		
Genetic dissection of late blight resistance in cultivated diploid Solanum species and modern potato varieties of Ecuador	Aug-Oct 2007	6.0
► **Writing a review or book chapter**		
► **MSc courses**		
PBR-20806 Plant Breeding	Sep-Oct 2007	3.0
ABG-30806 Modern Statistics for the Life Sciences	Jan-Feb 2008	3.0
Subtotal Start-Up Phase		13.5

2) Scientific Exposure	date	cp
► **EPS PhD student days**		
EPS PhD student day 2007, Wageningen, the Netherlands	13 Sep 2007	0.3
► **EPS theme symposia**		
EPS Theme 4 Symposium 'Genome Plasticity', Wageningen, the Netherlands	10 Dec 2010	0.3
EPS Theme 2 Symposium 'Interactions between Plants and Biotic Agents' & Willie Commelin Scholten Day, Amsterdam, the Netherlands	03 Feb 2011	0.3
► **Lunteren Days and other national platforms**		
Annual Meeting 'Experimental Plant Sciences', Lunteren, the Netherlands	04-05 Apr 2011	0.6
► **Seminars (series), workshops and symposia**		
European Flying Seminar: 'The Expanding Universe of Ubiquitin Fold Proteins', R. Vierstra	14 Apr 2008	0.1
European Flying Seminar: 'Integrating growth: a tale of two protons', S. Gilroy	19 May 2008	0.1
EPS Seminar: 'The hypochondriacs of the plant world: linking genetic diversification of the immune system to hybrid incompatibility', K. Bomblies	18 Nov 2010	0.1
EPS Seminar: 'Molecular basis of plant nutrition: Insights into the responses to magnesium and nitrate availability', C. Hermans	01 Dec 2010	0.1
Seminar Series Plant Sciences: 'Multi-scale assessment of climate change impact and adaptation', M. van Itersum	08 Feb 2011	0.1
Seminar Series Plant Sciences: 'Preparing crops for climate change: Breeding for abiotic stress tolerance', G. van der Linden	08 Feb 2011	0.1
Seminar Series Plant Sciences: 'GENERATION: Cultivating Plant Diversity for the Resource-Poor', J.M. Ribaut	31 May 2011	0.1
Seminar Series Plant Sciences: 'Career perspectives for young scientists' S. van der Ent & G. Schuber	14 Jun 2011	0.1
Symposium: 'Breeding Data: Statistical Advances in Modern Plant Breeding', Wageningen, the Netherlands	16 Oct 2018	0.3
► **Seminar plus**		
► **International symposia and congresses**		
III Congreso Nacional de la Papa (*III National Potato Congress*), Quito, Ecuador	18-20 Jun 2008	0.9

XXIII Congreso de la Asociación Latinoamericana de la Papa (*XXIII Congress of the Latin American Potato Association*), Mar del Plata, Argentina	30 Nov-06 Dec 2008	1.5
XLII Congresso Brasileiro de Fitopatologia (*XLII Brazilian Phytopathological Congress*), Rio de Janeiro, Brazil	03-07 Aug 2009	1.1
I Congreso Internacional de Investigacion y Desarrollo en Papas Nativas (*I International Congress on Research and Development of Native Potatoes*), Quito, Ecuador	18-20 Mar 2010	0.9
XXIV Congreso de la Asociación Latinoamericana de la Papa (*XXIV Congress of the Latin American Potato Association*), Cusco, Peru	23-28 May 2010	1.5
III Congreso Internacional de Biotecnología y Biodiversidad (*III International Biotechnology and Biodiversity Congress*), Guayaquil, Ecuador	10-13 Oct 2016	1.2
XXII Acorbat International Congress, Miami, USA	2-4 May 2018	0.9
► **Presentations**		
Poster: Selección de parentales resistentes al tizón tardío en especies diploides de Solanum spp. (*Selection of late blight resistant parental lines in Solanum spp. diploid accessions*) - III Congreso Nacional de la Papa, Quito, Ecuador	18-20 Jun 2008	1.0
Poster: Evaluación de resistencia al tizón tardio en introducciones diploides de Solanum spp. (*Evaluation of late blight resistance in Solanum spp. diploid accessions*) - XXIII Congreso de la Asociación Latinoamericana de la Papa, Mar del Plata, Argentina	30 Nov-05 Dec 2008	1.0
Poster: Ecuadorian native potatoes a source for late blight resistance - XLII Congresso Brasileiro de Fitopatologia, Rio de Janeiro, Brazil	03-07 Aug 2009	1.0
Poster: Caracterizacion de razas de Phytophthora infestans asociadas a papas nativas en la provincia del Carchi, Ecuador (*Characterization of races of Phytophthora infestans associated with native potatoes in Carchi province, Ecuador*) - I Congreso Internacional de Investigacion y Desarrollo de Papas Nativas, Quito, Ecuador	18-20 Mar 2010	1.0
Poster: Frecuencia de factores de virulencia de Phytophthora infestans en papas nativas en Carchi, Ecuador (*Frequency of Phytophthora infestans virulence factors in native potatoes in Carchi, Ecuador*) - XXIV Congreso de la Asociación Latinoamericana de la Papa, Cusco, Peru	23-28 May 2010	1.0
Talk: Evaluación de resistencia al tizón tardío en cultivares de papa. (*Evaluation of late blight resistance in potato cultivars*) - Taller en metodos aplicados en evaluaciones y analisis en la mejora genetica de la papa, Quito, Ecuador	22-26 Mar 2010	1.0
► **IAB interview**	21 Jan 2011	0.7
► **Excursions**		
Subtotal Scientific Exposure		17.3

3) In-Depth Studies		
	<u>date</u>	<u>cp</u>
► **Advanced scientific courses & workshops**		
Advanced Course in Modern Breeding Techniques, Ghent, Belgium	14-23 Aug 2007	3.0
Técnicas moleculares para el análisis de genomas (*Molecular techniques for genome analysis*), Quito, Ecuador	21-24 Oct 2008	1.3
Datos Multivariados: Análisis clásicos y nuevas tecnologías (*Multivariate data: Classical analysis and new technologies*), Turrialba, Costa Rica	22-26 Jun 2009	1.5
Uso de marcadores moleculares no melhoramento de plantas para resistencia a fitopatogenos (*Use of molecular markers in breeding for resistance to plant pathogens*), Rio de Janeiro, Brazil	04-06 Aug 2009	0.1
Taller en metodos aplicados en evaluaciones y analisis en la mejora genetica de la papa (*Workshop on applied methods for evaluation and analysis of genetic improvement of potatoes*), Quito, Ecuador	22-26 Mar 2010	1.3
Workshop on Plant Protection and Quarantine, International Cooperation and Development Fund (ICDF), Taipei, Taiwan	30 May-12 Jun 2012	3.0
Green chemicals and fuels, Guayaquil, Ecuador	02-07 Mar 2017	1.5
► **Journal club**		
Literature Discussion Meeting at Plant Breeding Group - Plant Research International	2008	0.4
► **Individual research training**		
Subtotal In-Depth Studies		12.1

4) Personal Development	*date*	*cp*
► **General skill training courses**		
Mini-Symposium: 'How to Write a World-Class Paper', Wageningen, the Netherlands	19 Apr 2011	0.2
PhD Scientific Writing, Wageningen, the Netherlands	11 May-29 Jun 2011	1.8
Preparation of competitive proposals for research and innovation in agriculture - IDB/INDES	01 Nov-11 Dec 2016	2.3
Webinar: 'Ethics in Scientific Publication', Jigisha Patel, Springer Nature	08 Dec 2016	0.1
► **Organisation of meetings, PhD courses or outreach activities**		
Member of scientific committee of the XXII Acorbat International Congress, Miami, USA	02-04 May 2018	1.5
► **Membership of EPS PhD Council**		

Subtotal Personal Development 5.9

TOTAL NUMBER OF CREDIT POINTS*	**48.8**

Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the Educational Committee of EPS with a minimum total of 30 ECTS credits.

** A credit represents a normative study load of 28 hours of study.*

This Research was financed by NUFFIC (Netherlands Organization for International Cooperation in Higher Education), Secretaría Nacional de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT) and National Agriculture Research Institute (INIAP) Quito-Ecuador.

Financial support from Wageningen University for printing this thesis is gratefully acknowledged.

Thesis layout and cover picture: Ricardo Delgado

Cover design by Dennis Hendriks

Printed by ProefschriftenMaken, The Netherlands.