The Population Genetic Structure of *Rhizoctonia solani* AG-3PT from Potato in the Colombian Andes

Rosa L. Ferrucho, Paulo C. Ceresini, Ursula M. Ramirez-Escobar, Bruce A. McDonald, Marc A. Cubeta, and Celsa García-Domínguez

First, third, and sixth authors: Facultad de Agronomía, Universidad Nacional de Colombia, Carrera 30 45-00 Bogotá, Colombia; second author: UNESP–Universidade Estadual Paulista, Campus de Ilha Solteira, Depto. de Fitossanidade, Engenharia Rural e Solos, 15385-000, Ilha Solteira, São Paulo, Brazil; fourth author: Institute of Integrative Biology–Plant Pathology, ETH Zurich, Zurich, Switzerland, 8092; and fifth author: Department of Plant Pathology, North Carolina State University, Raleigh 27695.
Accepted for publication 22 February 2013.

ABSTRACT

Ferrucho, R. L., Ceresini, P. C., Ramirez-Escobar, U. M., McDonald, B. A., Cubeta, M. A., and García-Domínguez, C. 2013. The population genetic structure of *Rhizoctonia solani* AG-3PT from potato in the Colombian Andes. *Phytopathology* 103:862–869.

The soilborne fungus *Rhizoctonia solani* anastomosis group 3 (AG-3PT) is a globally important potato pathogen. However, little is known about the population genetic processes affecting field populations of *R. solani* AG-3PT, especially in the South American Colombian Andes, which is near the center of diversity of the two most common groups of cultivated potato, *Solanum tuberosum* and *S. phureja*. We analyzed the

genetic structure of 15 populations of *R. solani* AG-3PT infecting potato in Colombia using 11 simple-sequence repeat (SSR) markers. In total, 288 different multilocus genotypes were identified among 349 fungal isolates. Clonal fractions within field populations were 7 to 33%. R_{ST} statistics indicated a very low level of population differentiation overall, consistent with high contemporary gene flow, though moderate differentiation was found for the most distant southern populations. Genotype flow was also detected, with the most common genotype found widely distributed among field populations. All populations showed evidence of a mixed reproductive mode, including both asexual and sexual reproduction, but two populations displayed evidence of inbreeding.

The basidiomycetous fungus *Rhizoctonia solani* Kühn anastomosis group 3 (AG-3PT) (teleomorph = *Thanatephorus cucumeris* (A. B. Frank) Donk) is a soilborne plant pathogen that can infect potato (*Solanum tuberosum* L.) and other solanaceous crops, including eggplant, pepper, and tomato (5,62). Infection of potato often occurs early in the growing season and results in killing of sprout tips and the development of cankers on stems (2,21,31). The disease delays and reduces the emergence of plants and decreases the number of stems. As the plant matures, stolons can be infected, resulting in the production of deformed tubers of reduced quality and market value (31). At the end of the growing season when the plants begin to senesce, the fungus forms black sclerotia (the black scurf phase of the disease) on the surface of daughter tubers (5). The fungus overwinters as mycelia and sclerotia in diseased plant tissue, in soil, and on tubers (2,5,21, 35,46). The sexual stage (forming basidia and basidiospores) can form during periods of high relative humidity at the base of potato stems in the plant canopy (35,62). However, the importance of sexual spores in the disease cycle of *R. solani* AG-3PT is not well understood.

Though *R. solani* AG-3PT is described as having a predominantly asexual lifestyle associated with the production of mycelia and sclerotia as the primary structures for dispersal and survival (13,14), population genetic studies reported variable *R. solani* AG-3PT populations that were not highly clonal (4,11,36). Field studies needed to determine the role of sexual reproduction

(implying a role for basidiospores) in the disease cycle are lacking. A population genetic study of five geographical populations of *R. solani* AG-3PT from eastern North Carolina found no subdivision, suggesting that long-distance gene flow was occurring (11). The lack of population differentiation was postulated to be a consequence of continuous introduction of tuber-borne inoculum from Canada and the northern United States into commercial potato-production fields in North Carolina (9–11).

There have been no studies on the population genetics of *R. solani* AG-3PT in South America, a region that is thought to be the center of origin for potato (7,56) and, possibly, of potato pathogens (27). Various researchers have proposed that potato plants were domesticated by the Bolivian natives in the region located between lakes Titicaca and Poopó (39,47,56) and subsequently dispersed throughout South America. The pre-Hispanic Muisca or Chibcha peoples living in the cold highlands of central Colombia cropped potato (named yomsa or yomuy) beginning 1,200 to 1,500 years ago (44,50). The potato was introduced into Europe from South America in 1567 (3,47). However, it was only after 1850 that potato became an important crop in the Colombian highlands (39) and a major agricultural commodity for export (52). The modern Colombian potato varieties originated from Colombian potato germplasm and were developed through local breeding efforts (16,22,61).

In Colombia, as in most of South America's potato-growing regions, *R. solani* AG-3PT is the most widespread soilborne pathogen affecting the two most common groups of potato, *S. tuberosum* L. and *S. phureja* (Juz. & Bukasov) Hawkesand (19). Seed tubers infested with mycelium and sclerotia of *R. solani* likely contribute to the widespread occurrence and dissemination of the pathogen in the region (5,23). The use of seed tubers certified to be free of *R. solani* could reduce long-distance dispersal of the pathogen; however, current phytosanitary regulations allow $\leq 10\%$ infestation of seed potato (33). Seed tubers are not

Corresponding author: P. C. Ceresini;
E-mail address: paulo.ceresini@bio.feis.unesp.br

*The e-Xtra logo stands for “electronic extra” and indicates that the online version contains two supplementary tables.

<http://dx.doi.org/10.1094/PHYTO-11-12-0278-R>
© 2013 The American Phytopathological Society

imported into Colombia, except in Departments close to Ecuador and Venezuela that are also in the Andes (16,33). Thus, the introduction of *R. solani* AG-3 on infected potato tubers from outside of Colombia is expected to be rare. An integrated approach including cultural, chemical, and biological control practices (5) is needed to manage this disease. In Colombia, only three fungicides (thiophanate-methyl and the mixture of carboxin + captan) are currently labeled for managing this disease on seed potato (34). There are no resistant varieties currently available, although earlier studies reported potato germplasm with differential responses to *R. solani* AG-3PT (8).

Despite the relative importance of black scurf and stem canker disease in Colombia, the pathogen biology remains poorly understood and questions related to the genetic diversity and structure of *R. solani* AG-3PT populations remain unanswered. The primary objective of this study was to ascertain the population genetic structure of potato-infecting *R. solani* AG-3PT from the Colombian Andes using recently developed simple-sequence repeat (SSR) genetic markers (20). The first objective was to determine whether populations were genetically subdivided or exhibited significant gene flow. Based on earlier findings described above, our null hypothesis was that there is no subdivision among geographical populations of *R. solani* AG-3 PT. The second objective was to determine whether the genetic structure in Colombia was consistent with a recombining population. Our null hypothesis was that populations would exhibit a mixed reproductive system characterized by random mating and local dispersal of a small number of clones. An alternative hypothesis was that populations would be largely asexual, with a few highly successful clones that are widely distributed across Colombia.

MATERIALS AND METHODS

Population sampling, fungal isolation, and DNA extraction.

Seven major potato-producing Departments in Colombia were sampled: Nariño and Cauca in the south, Antioquia in the west, Cundinamarca and Boyacá in the center, and Santander and Norte de Santander in the north (Table 1; Fig. 1). These Departments are responsible for approximately 85% of the potato production in Colombia (16). The sampling strategy was to obtain sympatric pairs of field populations from each county sampled in each Department. In three counties (Sibate, Carcasí, and Chitagá), it was not possible to obtain enough isolates from one of the paired fields to allow a meaningful comparison between fields within a county. But in six counties, we obtained complete paired collections. Naturally infected 2- to 3-month-old potato plants exhibiting symptoms of stem canker were sampled from 5 fields in

Cundinamarca, 4 fields in Boyacá, 2 fields in Antioquia, 2 fields in Nariño, 1 field in Santander, and 1 field in Norte de Santander, generating a total of 15 field populations (Table 1). In each field, transect sampling was used to collect 20 to 35 symptomatic plants. Seven transects were spaced 10 m apart, with five plants separated by 5 to 10 m sampled along each transect. From 10 field populations, we obtained 54 isolates from potato seed tubers (Table 2) to determine the genotype diversity associated with seedborne inoculum. We also included three populations of *R. solani* AG-3 from *S. phureja*, which is an important native diploid potato with a low level of domestication that is historically cropped in the area (22,61). Detailed field histories were not available but potato growers in the sampled areas generally rotate potato crops with pasture grasses which are not hosts for *R. solani* AG-3 (19).

Isolations were made by transferring fragments of infected stems or sclerotia to petri plates containing selective media (37) and incubating at 20°C in the dark. Hyphal tips from 1- or 2-day-old colonies were transferred to potato dextrose agar (PDA) (Oxoid Ltd., Hampshire, UK). Sclerotia from 25-day-old cultures growing on PDA plates were transferred to 1.8-ml cryotubes (Nunc CryoLine System, Roskilde, Denmark) containing anhydrous silica gel for long-term storage at 4°C. Stored sclerotia were used to initiate new cultures as required.

Mycelia for genomic DNA extraction were obtained from 5-day-old cultures on PDA containing a sterile cellophane membrane on top. After incubation at room temperature, mycelium was scraped from the cellophane membrane, then frozen and lyophilized. DNA was extracted from lyophilized mycelium with the DNeasy Plant Mini-Kit (Qiagen, Hilden, Germany). The anastomosis grouping of *R. solani* isolates was determined by polymerase chain reaction (PCR) using AG-3PT-specific primers for the internal transcribed spacer 5.8S region of the ribosomal DNA (38).

SSR genotyping. Isolates were genotyped using 11 polymorphic SSR loci using fluorescent-labeled primers (20). PCR conditions for all loci were those used by Ferrucho et al. (20), except for a final extension cycle of 30 min at 72°C. For fragment analyses, 1 to 2 µl of fluorescent-labeled PCR products were mixed and electrophoresed using an ABI PRISM 3100 (Applied Biosystems) automated sequencer at the Genetic Diversity Center of ETH Zurich along with a fluorescent-labeled size standard (GeneScan-500 LIZ; Applied Biosystems, Foster City, CA). Isolate RH002 (from tuber-borne sclerotia) was included as a positive control in every run of 94 samples. Additionally, we repeated the genotyping of population Cogua2, one of the two populations used for developing the SSR markers (20). Isolates from Boyacá,

TABLE 1. Description of the field populations of *Rhizoctonia solani* anastomosis group 3PT sampled from potato in the Colombian Andes that were included in this study^z

Department	County	Population name	Latitude	Longitude	Altitude (masl)	Linear distance (km)	Potato cultivar	Sampling year
Cundinamarca	Subachoque	Subachoque1	4°57'26.00"N	74°11'59.00"W	2931	0	<i>Solanum tuberosum</i> 'Diacol capiro'	2006
		Subachoque2	4°57'11.00"N	74°12'56.00"W	3095	2.3	<i>S. tuberosum</i> 'Diacol capiro'	2006
	Cogua	Cogua1	5°04'32.00"N	73°58'33.00"W	2730	28.0	<i>S. phureja</i>	2006
		Cogua2	5°04'22.00"N	73°58'45.00"W	2722	27.4	<i>S. phureja</i>	2006
		Sibate	4°25'16.00"N	74°14'56.00"W	3296	59.3	<i>S. tuberosum</i> 'Parda pastusa'	2006
Nariño	Pasto	Pasto1	1°8'41.50"N	77°20'29.29"W	3095	558.0	<i>S. tuberosum</i> 'Parda pastusa'	2007
		Pasto2	1°8'16.74"N	77°18'37.91"W	3084	546.0	<i>S. tuberosum</i> 'Parda pastusa'	2007
Antioquia	La Unión	LaUnion1	5°58'22.00"N	75°22'59.00"W	2505	172.1	<i>S. tuberosum</i> 'Diacol capiro'	2008
		LaUnion2	6°00'26.00"N	75°21'40.00"W	2504	173.5	<i>S. tuberosum</i> 'Diacol capiro'	2008
Boyacá	Ventaquemada	Ventaquemada1	5°23'30.40"N	73°27'49.10"W	2918	95.3	<i>S. tuberosum</i> 'Parda pastusa'	2007
		Ventaquemada2	5°25'00.20"N	73°27'25.30"W	2907	97.3	<i>S. tuberosum</i> 'ICA única'	2009
	Soraca	Soraca1	5°30'09.00"N	73°20'22.09"W	2809	114.0	<i>S. phureja</i>	2007
		Soraca2	5°30'45.00"N	73°19'33.00"W	2849	112.6	<i>S. tuberosum</i> 'Diacol capiro'	2009
Santander	Carcasí	Carcasí	6°04'47.00"N	72°33'01.81"W	3296	261.0	<i>S. tuberosum</i> 'Parda pastusa'	2008
Norte de Santander	Chitagá	Chitagá	7°03'06.20"N	72°40'06.38"W	2989	286.0	<i>S. tuberosum</i> 'Parda pastusa'	2008

^z Geographical coordinates are provided as latitude and longitude. Altitude is expressed in meters above sea level (masl). Mean linear distance between counties was calculated relative to the field Subachoque1. Distances ranged from 0.5 km between fields in the same county to 840 km between fields from the two most distant Departments.

Santander, and Norte de Santander Departments were processed in the Institute of Genetics of the Universidad Nacional de Colombia. The PCR conditions were as described previously. For fragment analyses, we used an ABI PRISM 3500 (Applied Biosystems) automated sequencer. Isolate RH002 was also included as a positive control in every run of 94 samples. As controls, 15 isolates genotyped at ETH were included in the fragment analysis conducted in Colombia. Statistical binning of the alleles was conducted using the software GeneMapper version 4.0 (Applied Biosystems).

Data analysis. SSR information content. Though it is dikaryotic, *R. solani* AG-3PT was treated as a functional diploid for all analyses, and all data were consistent with this assumption (11,36). The first analysis for the 11 SSR loci determined the range of repeats for each locus (Supplementary Table 1), the total and the average number of alleles per locus and per sampled field, and the identification of private alleles (i.e., those present in only one population) using allele frequency data computed by the program CONVERT version 1.31 (26).

Genotype diversity. Each isolate was assigned to a multilocus microsatellite genotype (MLMG) using GENODIVE (43). Isolates with the same MLMG were treated as clones. Indices of clonal diversity were calculated as follows: (i) number of MLMGs per population; (ii) site-specific MLMGs; (iii) clonal fraction or the proportion of isolates originating from asexual reproduction, calculated as $1 - (\text{number of different MLMGs})/(\text{total number of isolates})$ (65); (iv) Stoddart and Taylor's genotypic diversity, calculated as $G_0 = 1/\sum p_i^2$, where p_i is the frequency of the i th genotype (59); and (v) evenness, as an indicator of how the genotypes are distributed within the population (G_0 scaled by the maximum number of expected genotypes) (29). Comparisons for differences in clonal diversity indices among pairs of populations were performed using a pairwise bootstrap test (40) using 1,000 permutations, with subsampling to match the size of the smallest population (29). Finally, we performed the clone correction, selecting only one individual of each MLMG to perform subsequent analyses.

Gene diversity and population differentiation. Allelic richness and expected heterozygosity were calculated as indexes of gene diversity using the program FSTAT 2.9.3.2 (28). Nei's unbiased gene diversity or expected heterozygosity was estimated as $n/(n-1) \times (1 - \sum p_i^2)$, where p is the observed frequency of the i th allele

and n is the sample size (45). Allelic richness was estimated as the mean number of alleles per locus (15) for a standardized sample size of eight individuals, using rarefaction (32), as described previously (48). Finally, we tested whether pairs of populations differed for allelic richness and gene diversity with FSTAT version 2.9.3.2 using bootstrapping based on 1,000 permutations.

The distribution of gene diversity was evaluated using hierarchical analysis of molecular variance (AMOVA) (18). F statistics were calculated to quantify differentiation between pairs of populations and to assess the degree of population subdivision, based on the sum of squared size differences for SSR loci (R_{ST}) (55). The null distribution of pairwise F statistics values under the hypothesis of no differentiation between two populations was obtained by permuting haplotypes between populations. Genetic differentiation between populations was considered significant when $P \leq 0.05$. AMOVA was used to partition the covariance components due to among group (Departments), among-populations-within-group, and within-population effects. Significance of the F statistics was tested using 10,100 permutations by a non-parametric approach (18) using the program ARLEQUIN version 3.11 (17).

Reproductive mode: Hardy-Weinberg equilibrium and multilocus associations. To assess the contribution of sexual recombination to population structure, tests of Hardy-Weinberg Equilibrium (HWE) and multilocus association were performed. Tests for HWE were performed for each locus within each population with the program ARLEQUIN 3.11. This test is analogous to Fisher's exact test on a two-by-two contingency table but extended to a contingency table of arbitrary size (30,49). P values were obtained using a Markov Chain Monte Carlo (MCMC) approach, generating an exact probability distribution not biased by rare alleles or low sample size (49). The inbreeding coefficient (F_{IS}) across loci was calculated to test for a significant deficit or excess of heterozygotes when compared with HWE expectations (63), based on 10,100 permutations using the program ARLEQUIN 3.11.

The hypothesis of independence among alleles at distinct pairs of loci was tested using Fisher's exact test (25) based on an MCMC algorithm (with 10,000 batches and 10,000 iterations per batch) (49) and implemented in GENETPOP (49) version 3.4. To avoid type I error, the significance levels were adjusted using the Bonferroni correction (51). The multilocus index of association

Fig. 1. Geographic population samples of *Rhizoctonia solani* anastomosis group 3PT from potato in the Colombian Andes. **A**, Department and **B**, county populations. The latitude (north–south) and longitude (west–east) values are indicated on the maps.

(I_A) (41) and $\bar{r}_D$ (an alternative measure of I_A) were tested for significance with 10,000 randomizations (1). These tests are implemented in MULTILOCUS version 1.3 (1). An I_A significantly different from zero indicates linkage disequilibrium. $\bar{r}_D$ is a modified measure of I_A , adjusted for the number of loci. This index ranges from zero, when there is no disequilibrium, to one, when there is disequilibrium among tested loci.

RESULTS

SSR information content, gene and genotype diversity.

Eleven SSR loci were used to characterize the genetic structure of 15 populations of *R. solani* AG-3PT from the Colombian Andes. The SSR datasets were collected in two different laboratories, using different supplies and also two different automated fragment analyzers. The allele size data obtained for 15 control isolates and for population Cogua2 in the two laboratories were consistent, showing that the SSR markers were repeatable and reproducible between laboratories.

All SSR loci were polymorphic in all populations, except for TC_AG3_8, which was monomorphic in two populations (Subachoque2 and Pasto1). The number of alleles at each locus ranged from 2 (TC_AG3_0) to 14 (TC_AG3_16), with an average of 9 alleles per locus. The overall allelic richness across populations was 3.8. Nei's unbiased gene diversity was 0.56 to 0.65 across populations (Table 2). In total, 21 private alleles were found in 10 populations (Table 2).

We identified 288 MLMGs among the 349 isolates analyzed (Table 2). All 288 MLMGs were heterozygous for at least 1 of the 11 loci examined. In total, 246 individuals (85% of the population) were heterozygous for more than five loci, while 19 individuals (6.5% of the population) were heterozygous for nine loci. There were no significant differences between pairs of populations for either genotypic diversity or evenness. Low clonal frac-

tions were observed in most populations, with the highest clonal fractions found in Ventaquemada2 (0.29) and Subachoque2 (0.33). In total, 33 MLMGs representing 80 isolates were shared among field populations. Although MLMG 1 was sampled 11 times from distinct populations (this clone was found once in population Subachoque1, twice in Subachoque2 and Cogua2, and three times in La Unión1 and La Unión2), clones of most MLMGs were distributed mainly within the same field or county. Population Subachoque1 shared the largest number of MLMGs (11 of 23 isolates). Using the P_{sex} -value test statistic (57), we calculated the likelihood of observing MLMG 1 more than once across these five populations as a result of independent assortment of alleles following sexual reproduction. The P_{sex} statistic was 4.27^{-79} , making it highly unlikely that this shared genotype emerged independently in each population as a result of sexual reproduction.

Comparisons between pairs of isolates collected from different stems in the same plant showed that most of them (67% of the 36 pairs tested) represented a different MLMG, indicating that a single plant is often infected by more than one genotype of this pathogen. Comparing isolates obtained from stems and seed tubers from the same plant revealed that only 18% of the 56 pairs tested were clones, providing evidence for multiple infections on potato stems and seed tubers, which can be due to the distinct nature of the inoculum, either soil- or even seed-tuber-borne.

Population differentiation. AMOVA showed that variation among Departments and among fields within Departments each represented <1% of the total variance. In contrast, the variation within fields accounted for 94% of the total variance (Table 3). Pairwise analyses of population differentiation showed that most of the populations were very similar. There was no evidence for population differentiation between years. Among 104 pairwise comparisons, 22 were significantly differentiated ($P \leq 0.05$). Five of these pairs showed low differentiation ($R_{ST} < 0.05$) while 17 pairs were moderately differentiated (R_{ST} of 0.05 to 0.15) (Sup-

TABLE 2. Measures of gene and genotype diversity in field populations of the potato-infecting pathogen *Rhizoctonia solani* anastomosis group 3 PT from the Colombian Andes

State, population	Stems (N)	Tubers (N)	Total (N) ^s	Genotypes (N)	Site-specific genotypes ^t	Clonal fraction	Stoddart's G_o^u	Evenness ^v	$H_e^{w,x}$	Allelic richness ^{x,y}	Private alleles (N) ^z
Cundinamarca											
Subachoque1	17	11	28	23	12 (11)	0.18	19.60	0.85	0.62 a	3.65 b	0
Subachoque2	12	6	18	12	5 (7)	0.33	10.10	0.84	0.62 a	3.56 b	0
Cogua1	20	3	23	21	17 (4)	0.09	19.60	0.93	0.64 a	4.17 a	3
Cogua2	23	11	34	27	20 (7)	0.21	22.20	0.82	0.62 a	3.79 b	2
Sibate	26	7	33	28	23 (5)	0.15	25.30	0.90	0.63 a	4.28 a	3
Nariño											
Pasto1	18	0	18	17	16 (1)	0.06	16.20	0.95	0.57 b	3.63 b	1
Pasto2	22	0	22	19	16 (3)	0.14	16.10	0.85	0.56 b	3.73 b	1
Antioquia											
La Unión1	17	0	17	14	9 (5)	0.18	11.60	0.83	0.64 a	3.82 b	1
La Unión2	33	2	35	28	21 (7)	0.20	24.00	0.86	0.62 a	3.88 b	4
Boyacá											
Soraca1	21	1	22	18	14 (4)	0.18	16.10	0.90	0.57 b	3.75 b	0
Soraca2	22	1	23	17	11 (6)	0.26	12.90	0.76	0.63 a	4.08 a	2
Ventaquemada1	20	0	20	17	14 (3)	0.15	15.40	0.91	0.58 a	3.84 b	0
Ventaquemada2	21	0	21	15	10 (5)	0.29	13.40	0.89	0.58 b	3.40 c	0
Santander											
Carcasí	12	9	21	19	13 (6)	0.10	16.30	0.86	0.65 a	4.00 a	3
Norte de Santander											
Chitagá	11	3	14	13	7 (6)	0.07	12.30	0.94	0.56 b	3.46 c	1
Overall	295	54	349	288	208 (80)	0.17	16.74	0.87	0.61	3.80	21

^s Population sample size.

^t Number of genotypes shared with other populations are shown in parentheses. The total of 80 individuals shared among populations represented 33 distinct multilocus microsatellite genotypes, of which 26 genotypes were shared only once among populations and seven were shared more than once.

^u Stoddart and Taylor's genotypic diversity (58,59).

^v G_o scaled by the maximum number of expected genotypes; an evenness value = 1.0 indicates that all genotypes have equal frequencies.

^w Nei's unbiased gene diversity (45), also known as expected heterozygosity, averaged over all loci, corrected for sample size.

^x To test whether pairwise samples differed for Nei's unbiased gene diversity and allelic richness, we used FSTAT v.2.9.3.2 (28), based on 1,000 permutations. Means followed by the same letter are not significantly different at $P \leq 0.05$. Bold indicates the highest means.

^y Calculated according to El Mousadik and Petit (15), with subsampling to match the sample size of the smallest population.

^z Alleles occurring in only one population, determined with Convert 1.31 (26).

plementary Table 2). Populations in close geographical proximity (0.3 to 3.0 km distant from one another) had R_{ST} values close to zero. Populations located in southern Colombia (Nariño Department) were differentiated from populations sampled in Cundinamarca, Boyacá, Santander, and Norte de Santander Departments, which were 550 to 840 km apart. Pooling field populations from the same county into single-county populations supported previous results (Table 4). Pooling different county populations into Departments indicated that the most southern populations (Nariño Department) were genetically differentiated from the northern (Santander and Norte de Santander) and from the central (Cundinamarca and Boyacá) Departments. We found evidence for isolation by distance when comparing pairwise genetic distances [$\Phi ST/(1 - \Phi ST)$] and geographical distances [$\ln(D + 1)$, where D = average distance between Departments, in km]. The regression between genetic distances (Y) and geographical distances (X) was significant at $P = 0.004$ ($Y = -0.0302 + 0.0078X$; $R^2 = 0.41$) (Fig. 2). Populations of *R. solani* AG-3 from *S. phureja* and *S. tuberosum* were not significantly differentiated.

Reproductive mode: HWE and multilocus associations. The exact test for HWE showed that an average of 78% of loci were in HWE in the local populations (Table 5). Pasto1 deviated from HWE expectations in 4 of 10 loci examined. This population also showed a significant F_{IS} value (0.25, $P = 0.02$). A similar trend was observed for Soraca2, with four loci deviating from HWE and a F_{IS} value of 0.28 ($P = 0.01$), which is consistent with in-

breeding. F_{IS} values were nonsignificant for the remaining 13 populations. The overall F_{IS} value was 0.042 ($P = 0.076$). Locus TC_AG3_10 deviated from HWE in 8 populations while TC_AG3_9 and TC_AG3_6 deviated from HWE in 6 of 15 populations. Deviations from HWE were associated with a deficiency of heterozygotes in these populations.

Multilocus gametic equilibrium indices I_A and $\bar{r}_D$ were significantly different from zero ($P \leq 0.001$) for 10 of 15 populations (I_A of 0.33 to 1.39 and $\bar{r}_D$ of 0.034 to 0.14) (Table 5). However, only three populations showed >10% of locus pairs in disequilibrium: Subachoque1 (16.3%), Cogua2 (25.4%), and LaUnión2 (14.5%).

DISCUSSION

This is the first comprehensive study describing the genetic structure of potato-infecting populations of *R. solani* AG-3PT from cropping areas close to the putative center of origin of potato. Our sampling strategy included the most important potato-producing areas in the Colombian Andes. Comparisons were made between pairs of sympatric populations to understand the extent and distribution of genetic variation within counties and among more distant regional populations.

We hypothesize that gene and genotype flow make significant contributions to the population structure of this pathogen. Our results showed either no or very low pairwise population subdivision, consistent with significant gene flow among populations

TABLE 3. Hierarchical distribution of gene diversity among field populations of *Rhizoctonia solani* anastomosis group 3PT sampled from diseased potato in the Colombian Andes^z

Source of variation	Distance method: sum of squared size differences (R_{ST})					
	df	Sum of squares	Variance components	Variation (%)	Fixation indices	P value
Among Departments	4	599.6	0.61	0.99	$F_{CT} = 0.0099$	0.079
Among populations within Departments	10	833.7	0.52	0.84	$F_{SC} = 0.0085$	0.110
Among individuals within populations	273	17,379.4	2.85	4.59	$F_{IS} = 0.0468$	0.076
Within individuals	288	16,695.0	57.97	93.58	$F_{IT} = 0.0642$	0.025
Total	575	35,507.7	61.95	...	...	...

^z Analysis of molecular variance performed using the program ARLEQUIN version 3.11 (18). Distance method based on the sum of squared size differences (R_{ST}) between two haplotypes for microsatellite data, according to Slatkin (55). Number of permutations = 10,100. Groups correspond to the Departments sampled. Santander and Norte de Santander were pooled into a single group. P values show the probability of having, by chance alone, a more extreme variance component than the observed values.

TABLE 4. Fixation indices (R_{ST}) for pairwise comparisons between counties' or Departments' populations of *Rhizoctonia solani* anastomosis group 3PT sampled from diseased potato in the Colombian Andes^x

Contrasts			R_{ST}^y	P value
Between counties ^z				
Cundinamarca				
Sibate	versus	Subachoque (Cundinamarca)	0.0375	0.016
Sibate	versus	Ventaquemada (Boyacá)	0.0438	0.001
Sibate	versus	La Unión (Antioquia)	0.0286	0.021
Nariño				
Pasto	versus	Subachoque (Cundinamarca)	0.0712	0.001
Pasto	versus	Cogua (Cundinamarca)	0.0390	0.000
Pasto	versus	La Unión (Antioquia)	0.0330	0.010
Pasto	versus	Ventaquemada (Boyacá)	0.0718	0.000
Pasto	versus	Soraca (Boyacá)	0.0496	0.003
Pasto	versus	Carcasí (Santander)	0.0901	0.000
Pasto	versus	Chitagá (Norte de Santander)	0.0436	0.044
Between Departments				
Cundinamarca	versus	Nariño	0.024	0.033
Cundinamarca	versus	Boyacá	0.001	0.345
Cundinamarca	versus	Antioquia	0.001	0.380
Nariño	versus	Antioquia	0.022	0.329
Nariño	versus	Boyacá	0.058	0.003
Nariño	versus	Santander + Norte de Santander	0.067	<0.001

^x For between-county comparisons, the two field populations from the same county were pooled. For between-Departments comparisons multiple county populations were pooled. Distances were computed as the sum of squared size differences between two haplotypes, for microsatellite data (55).

^y Values in bold are significant at $P \leq 0.05$.

^z Only pairs of populations with significantly different R_{ST} are presented. P value calculated based on 10,100 permutations using the program ARLEQUIN version 3.11.

in the Colombian Andes. Lack of population subdivision was previously reported for populations of AG-3PT sampled in the southern United States and Denmark (11,36). The human-mediated movement of infested seed potato was postulated as the most plausible cause of this low differentiation because *R. solani* AG-3PT is efficiently dispersed on seed potato (54). Although approximately 30 potato varieties are recommended by the Colombian agricultural extension services, only 5 are commonly cultivated: Diacol capiro, Parda pastusa, Criolla (*S. phureja*), Pastusa Suprema, and Sabanera (16). The majority of our fungal isolates came from the first three varieties. Cropping the same potato

cultivars across regions would facilitate long-distance dispersal of seed tubers infested by mycelia and sclerotia because the trade of infested seed potato within Colombia allows $\leq 10\%$ black scurf infection on tuber surfaces (33).

We also found evidence for genotype flow among populations. Among the 33 MLMGs that were shared among populations, 18 were dispersed within a range of 0.3 km (between fields within a county) to 60 km (between counties). However, 15 MLMGs were found in populations separated by at least 100 km and ≤ 550 km (between Departments). The finding of significant genotype flow indicates that selection may also contribute to the low level of

Fig. 2. Regression between genetic and geographical distance among pairs of potato-infecting populations of *Rhizoctonia solani* anastomosis group 3PT from Colombia. The estimation of differentiation is from Slatkin's Φ_{ST} , an F_{ST} analogue assuming a stepwise mutation model (3). Linear relationship between pairwise values of $\Phi_{ST}/(1 - \Phi_{ST})$ and the natural logarithm of geographic distances ($D + 1$, in kilometers) between all population pairs.

TABLE 5. Tests for random association of alleles within each locus and between pairs of loci in field populations of *Rhizoctonia solani* anastomosis group 3PT in Colombia

State, county	Clone-corrected <i>N</i>	Loci (<i>N</i>) under HWE ^v	<i>F</i> _{IS} ^w	<i>P</i> value ^x	<i>I</i> _A ^y	$\bar{r}_D$	<i>P</i> value	Locus pairs ^z	Percent
Cundinamarca									
Subachoque1	23	9/11	-0.04	0.64	1.398	0.144	<0.001	9/55	16.36
Subachoque2	12	10/10*	0.14	0.20	1.259	0.143	<0.001	2/45	4.44
Cogua1	21	7/11	0.18	0.06	0.285	0.029	0.3100	1/55	1.82
Cogua2	27	8/11	0.00	0.49	1.411	0.143	<0.001	14/55	25.45
Sibate	28	5/11	0.06	0.25	0.331	0.034	0.0132	4/55	7.27
Nariño									
Pasto1	17	6/10*	0.25	0.02	0.776	0.088	<0.001	0/45	0.00
Pasto2	19	9/11	0.09	0.20	0.650	0.067	0.0020	0/55	0.00
Antioquia									
LaUnión1	14	9/11	-0.03	0.56	1.176	0.123	<0.001	3/55	5.45
LaUnión2	28	8/11	-0.03	0.59	0.886	0.092	<0.001	8/55	14.55
Boyacá									
Soraca1	18	8/11	0.11	0.20	0.373	0.039	0.2650	0/51	0.00
Soraca2	17	7/11	0.28	0.01	0.513	0.052	0.0530	0/55	0.00
Ventaquemada1	17	8/11	-0.03	0.60	1.003	0.103	<0.001	0/55	0.00
Ventaquemada2	15	11/11	-0.11	0.73	1.294	0.134	<0.001	0/55	0.00
Santander									
Carcasí	19	9/11	-0.05	0.65	0.796	0.080	<0.001	1/55	1.82
Norte de Santander									
Chitagá	13	9/11	-0.19	0.85	0.933	0.098	<0.001	0/55	0.00

^v Number of loci under Hardy Weinberg equilibrium (HWE) test performed according to an exact test analogous to Fisher's exact test, using a Markov chain with forecast length of 10,100 (30); * indicates one monomorphic loci.

^w Population-specific *F*_{IS} indices and *P* values calculated based on 10,100 permutations using ARLEQUIN 3.11 (17).

^x Index of multilocus gametic disequilibrium (*I*_A) (for the random association of alleles among distinct loci pairs) (41). $\bar{r}_D$ is a modified measure of *I*_A, adjusted for the number of loci; its values range from zero to one.

^y Statistical significance for complete panmixia based on 10,000 randomizations; for diploid data, the two alleles at a locus are shuffled together (associations between alleles at a locus are maintained in the randomized data sets). This test is purely for associations between loci (1).

^z Number of pairs of loci with significant disequilibrium according to Fisher's exact test (probability test) using a Markov chain with 1,000 batches and 1,000 iteration/batch, implemented by Genepop (49), after Bonferroni correction for multiple comparisons (51).

population differentiation. The extensive cropping of relatively few potato cultivars could lead to selection for host-specialized pathogen clones that could be transmitted among fields on infested seed potato. It also is possible that similar climatic conditions across the range of agroecosystems where potato are cultivated in the Colombian Andes (2,500 to 3,100 m above sea level) could have selected for climatically specialized genotypes.

Subdivision was greatest between the most geographically distant populations in Nariño and the remaining populations, with R_{ST} values of 0.04 to 0.11 ($P \leq 0.05$). Because populations in Nariño are geographically more distant, long-distance dispersal of the fungus via seed tuber trading might have been less frequent. It also is possible that potato growers from this region have not been importing seed tubers for a long period or may have imported from a different source of seed tubers.

Though we compared populations of *R. solani* AG-3 PT from *S. tuberosum* and the local native potato *S. phureja* (61), including more populations from wild potato may have allowed us to infer a center of origin for the pathogen. However, finding areas of undisturbed wild vegetation in the Colombian Andes has become difficult because the natural landscape in these highlands has been largely converted to extensive pastures and agricultural areas. Interestingly, the native *S. phureja* potato and the *S. tuberosum* potato are infected by highly similar populations of *R. solani* AG-3 PT. Given the significant gene and genotype flow that we detected, it is likely that the domesticated and wild potato plants have been colonized by the same pathogen population.

Despite the extensive regional dispersal of this tuber-borne pathogen across the Colombian Andes, from our study, we cannot assert that the Colombian populations of *R. solani* AG-3 PT are a mixture between resident and introduced populations or represent a resident population of the pathogen. Three circumstances would favor the hypothesis of a predominantly resident population: (i) historical records indicated that potato domestication and small-scale potato cropping occurred locally in the Andean region long before the arrival of the European conquistadors in South America in the early 16th century (44,50), (ii) current potato cultivation is based largely on relatively few locally bred potato cultivars (16, 22,61), and (iii) external introductions of seed-potato in Colombia are expected to be rare (16,33). Under such circumstances, the signal of diversity and allelic richness associated with a resident population of the pathogen would persist locally. However, testing the hypothesis of a resident population would require extensive sampling of *R. solani* AG-3 from major potato-production areas to contrast the allele diversity observed in Colombia with other populations worldwide.

We also tested the hypothesis that the potato-infecting populations of *R. solani* AG-3PT are recombining, as has been shown for populations from the southern United States (11). The finding of high genotypic diversity coupled with high proportions of loci in HWE and gametic equilibrium indicate that most populations of *R. solani* AG-3PT in Colombian potato fields are recombining. F_{IS} values suggested that Pasto1 and Soraca2 have experienced some inbreeding. Asexual reproduction also made a significant contribution to population structure in every field, with a clonal fraction averaging 17% and some clones being widely distributed among populations. These findings of a mixed reproductive system are consistent with an "epidemic population structure", originally described for bacteria (41) but later extended to fungi (60). Analyses of the population genetic structure of the rice- and soybean-infecting pathogen *R. solani* AG-1 IA sampled over a large geographic scale also indicated a reproductive mode varying from strictly recombining to a mixed reproductive system, in which there are recombination events followed by clonal expansion during the growing season (6,12,53).

In conclusion, our study indicated that the population genetic structure of *R. solani* AG-3PT from the Colombian Andes has been shaped by human-mediated gene and genotype flow and by

a mixed reproductive system in which recombination events are followed by clonal multiplication and dispersal of a few well-adapted clones. Local populations are characterized by high gene and genotypic diversity and a relatively low clonal fraction. There was evidence for weak geographical differentiation among the most distant populations and little evidence for either host specialization or agroecosystem adaptation. The high gene and genotypic diversity combined with evidence for a mixed reproductive mode coupled with high gene and genotype flow among populations are characteristics of pathogens with high evolutionary potential (42,64). Pathogens with high evolutionary potential require special attention when formulating management strategies. In this case, over-reliance on fungicide applications should be avoided, especially considering that thifluzamide, the fungicide used in Colombia for the management of this disease (34), belongs to a group with medium to high risk of resistance (24). Given our current knowledge, it appears likely that fungicide-resistant strains could be rapidly disseminated across Colombia after they emerge.

ACKNOWLEDGMENTS

This research was supported by Universidad Nacional de Colombia, Ministerio de Agricultura y Desarrollo Rural de Colombia (MADR), Asociación Hortifrutícola de Colombia (Asohofrucol), and ETH Zurich (Swiss Federal Institute of Technology, Zurich, Switzerland). This work was partially funded by an ETH grant (TH-16/06-1) to P. C. Ceresini and a MADR-Asohofrucol grant to R. L. Ferrucho. P. C. Ceresini is a Research Fellow Pq2 from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil, grants 308394/2009-7 and 307361/2012-8). R. L. Ferrucho and P. C. Ceresini contributed equally to this article. Populations collected in Cundinamarca, Antioquia, and Nariño were genotyped at the Genetic Diversity Center of ETH. We thank M. Zala (ETH Zurich) for his excellent technical assistance.

LITERATURE CITED

1. Agapow, P. M., and Burt, A. 2001. Indices of multilocus linkage disequilibrium. *Mol. Ecol. Notes* 1:101-102.
2. Agrios, G. N. 2005. *Plant Pathology*, 5th. ed. Elsevier Academic Press, Burlington, MA.
3. Ames, M., and Spooner, D. M. 2008. DNA from herbarium specimens settles a controversy about origins of the European potato. *Am. J. Bot.* 95:252-257.
4. Balali, G. R., Neate, S. M., Kasalkheh, A. M., Stodart, B. J., Melanson, D. L., and Scott, E. S. 2007. Intraspecific variation of *Rhizoctonia solani* AG 3 isolates recovered from potato fields in Central Iran and South Australia. *Mycopathologia* 163:105-115.
5. Banville, G. J., Carling, D. E., and Ostysko, B. E. 1996. *Rhizoctonia* disease on potato. Pages 321-330 in: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology, and Disease Control. Kluwer Academic Publishers, Dordrecht, The Netherlands.
6. Bernardes-de-Assis, J., Storari, M., Zala, M., Wang, W., Jiang, D., ShiDong, L., Jin, M., McDonald, B. A., and Ceresini, P. C. 2009. Genetic structure of populations of the rice-infecting pathogen *Rhizoctonia solani* AG-1 IA from China. *Phytopathology* 99:1090-1099.
7. Brush, S., Kesseli, R., Ortega, R., Cisneros, P., Zimmerer, K., and Quiros, C. 1995. Potato diversity in the Andean center of crop domestication. *Conserv. Biol.* 9:1189-1198.
8. Buritica, C. P. E. 1975. Fuentes de resistencia en papa (*Solanum tuberosum* L.) a *Rhizoctonia solani* Khun. *Rev. ICA* 10:446.
9. Ceresini, P. C., Shew, H. D., James, T. Y., Vilgalys, R. J., and Cubeta, M. A. 2007. Phylogeography of the solanaceae-infecting Basidiomycota fungus *Rhizoctonia solani* AG-3 based on sequence analysis of two nuclear DNA loci. *BMC Evol. Biol.* 7:163.
10. Ceresini, P. C., Shew, H. D., Vilgalys, R. J., Gale, L. R., and Cubeta, M. A. 2003. Detecting migrants in populations of *Rhizoctonia solani* anastomosis group 3 from potato in North Carolina using multilocus genotype probabilities. *Phytopathology* 93:610-615.
11. Ceresini, P. C., Shew, H. D., Vilgalys, R. J., Rosewich, U. L., and Cubeta, M. A. 2002. Genetic structure of populations of *Rhizoctonia solani* AG-3 on potato in eastern North Carolina. *Mycologia* 94:450-460.
12. Ciampi, M. B., Meyer, M. C., Costa, M. N., Jr., Zala, M., McDonald, B. A., and Ceresini, P. C. 2008. Genetic structure of populations of *Rhizoctonia solani* anastomosis group-1 IA from soybean in Brazil. *Phyto-*

- pathology 98:932-941.
13. Cubeta, M. A., and Vilgalys, R. 1997. Population biology of the *Rhizoctonia solani* complex. *Phytopathology* 87:480-484.
 14. Cubeta, M. A., and Vilgalys, R. 2000. *Rhizoctonia*. Pages 109-116 in: *Encyclopedia of Microbiology*. J. Lederberg, M. Alexander, B. Bloom, D. Hopwood, R. Hull, B. Iglewski, A. Laskin, S. Oliver, M. Schaechter, and W. Summers. Academic Press, New York.
 15. El Mousadik, A., and Petit, R. J. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theor. Appl. Genet.* 92:832-839.
 16. Espinal, C. F., Martínez, H., Pinzón, N., and Barrios, C. 2006. La cadena de la papa en Colombia. Una mirada global de su estructura y dinámica 1991-2005. *Observatorio Agrociencias Colombia*, ed. Ministerio de Agricultura y Desarrollo Rural, Bogotá, Colombia.
 17. Excoffier, L., Laval, G., and Schneider, S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinf.* 1:47-50.
 18. Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131:479-491.
 19. Ferrucho, R. L., Cifuentes Castellanos, J. M., Ceresini, P. C., and García-Domínguez, C. 2012. *Rhizoctonia solani* AG-3PT is the major pathogen associated with potato stem canker and black scurf in Colombia. *Agron. Colomb.* 30:204-213.
 20. Ferrucho, R. L., Zala, M., Zhang, Z., Cubeta, M. A., García-Domínguez, C., and Ceresini, P. C. 2009. Highly polymorphic in silico-derived microsatellite loci in the potato-infecting fungal pathogen *Rhizoctonia solani* anastomosis group 3 from the Colombian Andes. *Mol. Ecol. Resour.* 9:1013-1016.
 21. Fox, R. 2006. *Rhizoctonia* stem and stolon canker of potato. *Mycologist* 20:116-117.
 22. Franco-Lara, L., and Barker, H. 1999. Characterization of resistance to potato leafroll virus accumulation in *Solanum phureja*. *Euphytica* 108:137-144.
 23. Frank, J. A., and Leach, S. S. 1980. Comparison of tuberborne and soilborne inoculum in the *Rhizoctonia* disease of potato. *Phytopathology* 70:51-53.
 24. Fungicide Resistance Action Committee (FRAC). 2012. FRAC Code List *2012: Fungicides sorted by mode of action (including FRAC code numbering). FRAC. <http://www.frac.info/frac/publication/anhang/FRAC-Code-List2011-final.pdf>
 25. Garnier-Gere, P., and Dillmann, C. 1992. A computer program for testing pairwise linkage disequilibria in subdivided populations. *J. Hered.* 83:239-239.
 26. Glaubitz, J. C. 2004. Convert: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Mol. Ecol. Notes* 4:309-310.
 27. Gómez-Alpizar, L., Carbone, I., and Ristaino, J. B. 2007. An Andean origin of *Phytophthora infestans* inferred from mitochondrial and nuclear gene genealogies. *Proc. Natl. Acad. Sci. USA* 104:3306-3311.
 28. Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate *F*-statistics. *J. Hered.* 86:485-486.
 29. Grünwald, N. J., Goodwin, S. B., Milgroom, M. G., and Fry, W. E. 2003. Analysis of genotypic diversity data for populations of microorganisms. *Phytopathology* 93:738-746.
 30. Guo, S. W., and Thompson, E. A. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* 48:361-372.
 31. Hide, G. A., and Horrocks, J. K. 1994. Influence of stem canker (*Rhizoctonia solani* Kuhn) on tuber yield, tuber size, reducing sugars and crisp colour in cv. Record. *Potato Res.* 37:43-49.
 32. Hurlbert, S. H. 1971. The non-concept of species diversity: a critique and alternative parameters. *Ecology* 52:577-586.
 33. Instituto Colombiano Agropecuario (ICA). 2003. Resolución 2501 por la cual se establecen los requisitos específicos mínimos para la producción de semilla certificada de papa. ICA, Bogotá, Colombia.
 34. Instituto Colombiano Agropecuario (ICA). 2011. Registros de venta de plaguicidas químicos de uso agrícola. <http://www.ica.gov.co/Areas/Agricola/Servicios/Regulacion-y-Control-de-Plaguicidas-Quimicos.aspx>
 35. Jeger, M. J., Hide, G. A., Boogert, P. H. J. F., Termorshuizen, A. J., and Baarlen, P. 1996. Pathology and control of soil-borne fungal pathogens of potato. *Potato Res.* 39:437-469.
 36. Justesen, A. F., Yohalem, D., Bay, A., and Nicolaisen, M. 2003. Genetic diversity in potato field populations of *Thanatephorus cucumeris* AG-3, revealed by ITS polymorphism and RAPD markers. *Mycol. Res.* 107:1323-1331.
 37. Ko, W. H., and Hora, F. 1971. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. *Phytopathology* 61:707-710.
 38. Lees, A. K., Cullen, D. W., Sullivan, L., and Nicolson, M. J. 2002. Development of conventional and quantitative real-time PCR assays for the detection and identification of *Rhizoctonia solani* AG-3 in potato and soil. *Plant Pathol.* 51:293-302.
 39. Luján, L. 1996. Historia de la papa. *Rev. Papa* 1:1-26.
 40. Manly, B. F. J. 1991. Randomization, Bootstrap and Monte Carlo Methods in Biology. Texts in Statistical Science, 2nd ed. C. Chatfield and J. Zidek, eds. Chapman & Hall/CRC, London.
 41. Maynard Smith, J., Smith, N. H., O'Rourke, M., and Spratt, B. G. 1993. How clonal are bacteria? *Proc. Natl. Acad. Sci. USA* 90:4384-4388.
 42. McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. *Annu. Rev. Phytopathol.* 40:349-379.
 43. Meirmans, P. G., and Van Tienderen, P. H. 2004. Genotype and Genodive: Two programs for the analysis of genetic diversity of asexual organisms. *Mol. Ecol. Notes* 4:792-794.
 44. Molano, J. 1995. Paisajes de la alta montaña ecuatorial. In: *El Páramo. Ecosistema de Alta Montaña*. Fundación Ecosistemas Andinos-ECOAN, Bogotá, D.C.
 45. Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583-590.
 46. Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intra-specific groups of *Rhizoctonia solani* Kuhn. *Annu. Rev. Phytopathol.* 25:125-143.
 47. Ovchinnikova, A., Krylova, E., Gavrilenko, T., Smekalova, T., Zhuk, T., Knapp, S., and Spooner, D. M. 2011. Taxonomy of cultivated potatoes (*Solanum* section Petota: Solanaceae). *Bot. J. Linn. Soc.* 165:107-155.
 48. Petit, R. J., El Mousadik, A., and Pons, O. 1998. Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.* 12:844-855.
 49. Raymond, M., and Rousset, F. 1995. Genepop (Version 1.2): Population genetics software for exact tests and ecumenicism. *J. Hered.* 86:248-249.
 50. Restrepo, V. 2013. Los chibchas antes de la conquista española. Bogotá: Imprensa de la luz, 1895. <http://www.banrepcultural.org/blaavirtual/historia/chibch/chibch11a.htm>
 51. Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223-225.
 52. Romans, A. 2005. The Potato Book. Frances Lincoln Ltd., London.
 53. Rosewich, U. L., Pettway, R. E., McDonald, B. A., and Kistler, H. C. 1999. High levels of gene flow and heterozygote excess characterize *Rhizoctonia solani* AG-1 IA (*Thanatephorus cucumeris*) from Texas. *Fungal Genet. Biol.* 28:148-159.
 54. Simons, S. A., and Gilligan, C. A. 1997. Relationships between stem canker, stolon canker, black scurf (*Rhizoctonia solani*) and yield of potato (*Solanum tuberosum*) under different agronomic conditions. *Plant Pathol.* 46:651-658.
 55. Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139:457-462.
 56. Spooner, D. M., McLean, K., Ramsay, G., Robbin, W., and Bryan, G. J. 2005. A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proc. Natl. Acad. Sci. USA* 102:14694-14699.
 57. Stenberg, P., and Lundmark, M. S. A. 2003. MLG_{SIM}: A program for detecting clones using a simulation approach. *Mol. Ecol. Notes* 3:329-331.
 58. Stoddart, J. A. 1983. A genotypic diversity measure. *J. Hered.* 74:489-490.
 59. Stoddart, J. A., and Taylor, J. F. 1988. Genotypic diversity: Estimation and prediction in samples. *Genetics* 118:705-711.
 60. Taylor, J., Jacobson, D., and Fisher, M. 1999. The evolution of asexual fungi: Reproduction, Speciation and Classification. *Annu. Rev. Phytopathol.* 37:197-246.
 61. Theisen, K. 2013. World potato atlas: Colombia chapter. Lima: International Potato Center, 2006. <https://research.cip.cgiar.org/confluence/display/wpa/Colombia>
 62. Tsror, L. 2010. Biology, epidemiology and management of *Rhizoctonia solani* on potato. *J. Phytopathol.* 158:649-658.
 63. Weir, B. S., and Cockerman, C. C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38:1358-1370.
 64. Willi, Y., Frank, A., Heinzelmann, R., Kalin, A., Spalinger, L., and Ceresini, P. C. 2011. The adaptive potential of a plant pathogenic fungus, *Rhizoctonia solani* AG-3, under heat and fungicide stress. *Genetica* 139:903-908.
 65. Zhan, J., Pettway, R. E., and McDonald, B. A. 2003. The global genetic structure of the wheat pathogen *Mycosphaerella graminicola* is characterized by high nuclear diversity, low mitochondrial diversity, regular recombination, and gene flow. *Fungal Genet. Biol.* 38:286-297.