Plant Pathology (2018) Doi: 10.1111/ppa.12880

Molecular, morphological and pathogenic diversity of *Sclerotinia sclerotiorum* isolates from common bean (*Phaseolus vulgaris*) fields in Argentina

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White mould, caused by *Sclerotinia sclerotiorum*, is one of the most threatening fungal diseases occurring across major bean production regions worldwide. In Argentina, under favourable weather conditions, up to 100% seed yield losses occur on susceptible common bean cultivars. The aim of this study was to characterize the diversity of *S. sclerotiorum* isolates from six dry bean fields in the main production area of Argentina by means of molecular, morphological (mycelium colour, number and pattern of sclerotia distribution) and pathogenic approaches. Among 116 isolates analysed, high genotypic and morphological variability was observed. A total of 52 mycelial compatibility groups (MCGs) and 59 URPs (universal rice primers) molecular haplotypes were found. All the MCGs were location specific, while only 12% of the URP haplotypes were shared among locations. The molecular analysis of variance revealed a significant differentiation among populations, with higher genetic variability within the populations analysed than among them. The aggressiveness of the isolates towards bean seedlings was assessed in the greenhouse. Most of the isolates were highly aggressive, while no variation among locations was observed. The information generated in the present study provides, for the first time, information on the variability of *S. sclerotiorum* associated with white mould in the main common bean production area in Argentina. In addition, the findings suggest the occurrence of both clonal and sexual reproduction in the populations analysed. This work contributes to the development of sustainable management strategies in bean production aimed to minimize yield losses due to white mould.

Keywords: aggressiveness, ITS sequencing, mycelial compatibility group, URP, white mould

Introduction

Common bean (*Phaseolus vulgaris*) is the most important food legume worldwide and represents the main source of protein and carbohydrate in the human diet of many countries in Africa and Latin America (Broughton *et al.*, 2003). Argentina exports 97% of its production and it is among the five major exporters of common bean worldwide (Vizgarra *et al.*, 2016). The main cultivation areas are concentrated in the provinces of Salta, Jujuy, Tucumán, Catamarca and Santiago del Estero, in the northwestern region of Argentina. Salta produces 73% of the total common bean production of the country (Ploper *et al.*, 2016), mainly in the departments of General San Martín and Orán in the north of the province (Paoli *et al.*, 2017). The main varieties produced are alubia white beans (41%) and black beans (42%); other varieties of minor relevance (17%) are cranberry, dark and light red kidney.

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White mould, caused by *Sclerotinia sclerotiorum*, is one of the most threatening fungal diseases occurring across major bean production regions of Europe, North and South America (Schwartz & Steadman, 1989), Australia (Sexton & Howlett, 2004) and in some African and Asian countries (Allen, 1983). White mould has been detected in all common bean production areas in northwestern Argentina, as well as in other crops, although disease incidence and severity vary depending on the region and the environmental conditions of each agricultural season. Under favourable weather conditions, 100% seed yield and quality losses occur on susceptible common bean cultivars (Singh & Schwartz, 2010). The department of San Martín in the north of Salta, with a conducive environment characterized by moderate to high temperatures and high humidity, is the area where white mould is most prevalent (Vizgarra *et al.*, 2016). Because commercial varieties with white mould resistance are not available, the disease is managed with a combination of approaches including fungicides during flowering, increased row spacing, deep ploughing and

the use of upright cultivars. Although crop rotation with nonhost crops is a common cultural practice to reduce white mould severity and incidence (Schwartz & Singh, 2013), monoculture prevails in the region.

Assessing the genetic diversity and reproductive behaviour of the white mould pathogen in a particular geographical region is important for breeding programmes that aim to develop new resistant cultivars. Several studies based on molecular markers and mycelial compatibility groupings revealed diversity in *S. sclerotiorum* genotypes and provided evidence of both clonal and sexual (out-crossing) reproduction in populations in North America, Australia and Brazil (Cubeta *et al.*, 1997; Sexton & Howlett, 2004; Gomes *et al.*, 2011; Lehner & Mizubuti, 2017). Some reports have mentioned that clonal reproduction is predominant in temperate agricultural zones, whereas sexual recombination contributes to population structure in tropical or subtropical climates (Cubeta *et al.*, 1997; Malvárez *et al.*, 2007). However, the high variability observed in the latter regions may be due to an increase in the number of studies performed with SSR markers in these regions (Lehner & Mizubuti, 2017). Moreover, a recent study compared *S. sclerotiorum* populations from different climatic zones from the USA and Brazil using microsatellite markers and mycelial compatibility groupings, and suggested that there was no relationship between the climate of origin and the evidence of out-crossing or the amount of diversity in the populations (Lehner *et al.*, 2017).

Furthermore, variation in aggressiveness among *S. sclerotiorum* isolates from common bean (Otto-Hanson *et al.*, 2011) and other hosts has been reported (Kull *et al.*, 2004; Sexton & Howlett, 2004). Isolate aggressiveness was shown to vary within widely dispersed mycelial compatibility groups. However, no variation in aggressiveness was observed in mycelial compatibility groups detected within single fields (Kull *et al.*, 2004).

Universal rice primers (URPs; Kang *et al.*, 2002) are dominant molecular markers that have been extensively used in fingerprinting studies for many pathogens (González *et al.*, 2012; Mann *et al.*, 2014). This technique has the advantage of being cost effective with no prior knowledge of the species genome needed for their use. Moreover, URP markers are more reproducible than those of RAPD (random amplified polymorphic DNA) because stringent PCR conditions with high annealing temperatures and longer primers are employed (Kang *et al.*, 2002). Also, because they have multiple polymorphic loci, URP markers are suitable for the analysis of closely related individuals (Kang *et al.*, 2002; González *et al.*, 2012; Mann *et al.*, 2014). A previous study has considered the utility of URP markers for the analysis of *S. sclerotiorum* isolates; however, few markers were used (Manjunatha *et al.*, 2014).

Estimating the genetic diversity of a pathogenic population in a region is important in order to find the best disease control strategy. Despite the relevance of white mould epidemics to Argentinean agriculture, no studies to investigate the population diversity of *S. sclerotiorum* have been performed. Thus, the aim of this study was to characterize the genetic and pathogenic diversity of the *S. sclerotiorum* isolates from common bean white mould in northwestern Argentina.

Materials and methods

Fungal isolates

Sclerotinia sclerotiorum isolates were obtained from sclerotia collected from common bean plants with white mould symptoms in six fields from Salta and Jujuy provinces, Argentina, during May 2014. The fields were located in six locations in the northwestern region of the country (Fig. 1). Samples were taken in a W pattern along the length of the field, and approximately 20 plants were selected per field. Sampled plants were representatives of white and black bean cultivars that are grown along the sampled area. The sampled cultivars were Alubia Cerrillos, PF1, Leales 10, Leales 24 and Leales 15. Distances between fields were between 15 and 156 km. Each isolate was derived from a single sclerotium, with only one isolate obtained from each plant. Sclerotia were surface-sterilized by immersion (70% ethanol for 2 min, 3% sodium hypochlorite solution for 1 min) then rinsed three times with sterile distilled water and blotted dry on sterile filter paper, in sterilized paper towels. Sclerotia were then bisected and transferred aseptically to potato dextrose agar (PDA) plates and incubated at 20–22 °C. After 72 h, each isolate was purified by hyphal tip isolation and placed onto new PDA plates for 2 weeks in the dark at room temperature. Sclerotia formed in culture were harvested, air dried and stored in paper bags at 4 °C as a stock for future use. Isolates collected in the same geographic region were considered as belonging to the same population.

Morphological characterization

Isolates were morphologically characterized after incubation on PDA at 20 ± 2 °C in darkness for 15 days. The mycelium colour, the pattern of sclerotia distribution and number of sclerotia developed in Petri dishes were recorded for each isolate. Each isolate was evaluated in three replicates.

Mycelial compatibility groups

Mycelial compatibility grouping (MCG) was performed by pairing each isolate in all combinations, including self–self, on PDA plates amended with red food colouring (Schafer & Kohn, 2006). Mycelial discs (5 mm diameter) were cut off from the edge of an actively growing colony and placed triangularly (4 cm from each other) in a 9 cm diameter PDA Petri plate amended with 175 μL L^{-1} of red food colouring. All pairings were scored after 7 and 14 days of incubation in the dark at room temperature (20–22 °C). Each pairing was performed three times. As a control for compatibility, each isolate was paired with itself. Pairings were scored as compatible when two strains merged to form one colony, with no distinct interaction zone, in which case the isolates were placed into the same group. Pairings were scored as incompatible when a thin-to-wide band of aerial mycelium on the surface accompanied by a thin red line on the bottom side of the Petri dish was observed between confronting paired isolates. The results were recorded in a binary matrix, as either an incompatible (0) or a compatible (1) reaction for each isolate pair. A randomized complete block design with two replications was used. The Shannon index, H_0,

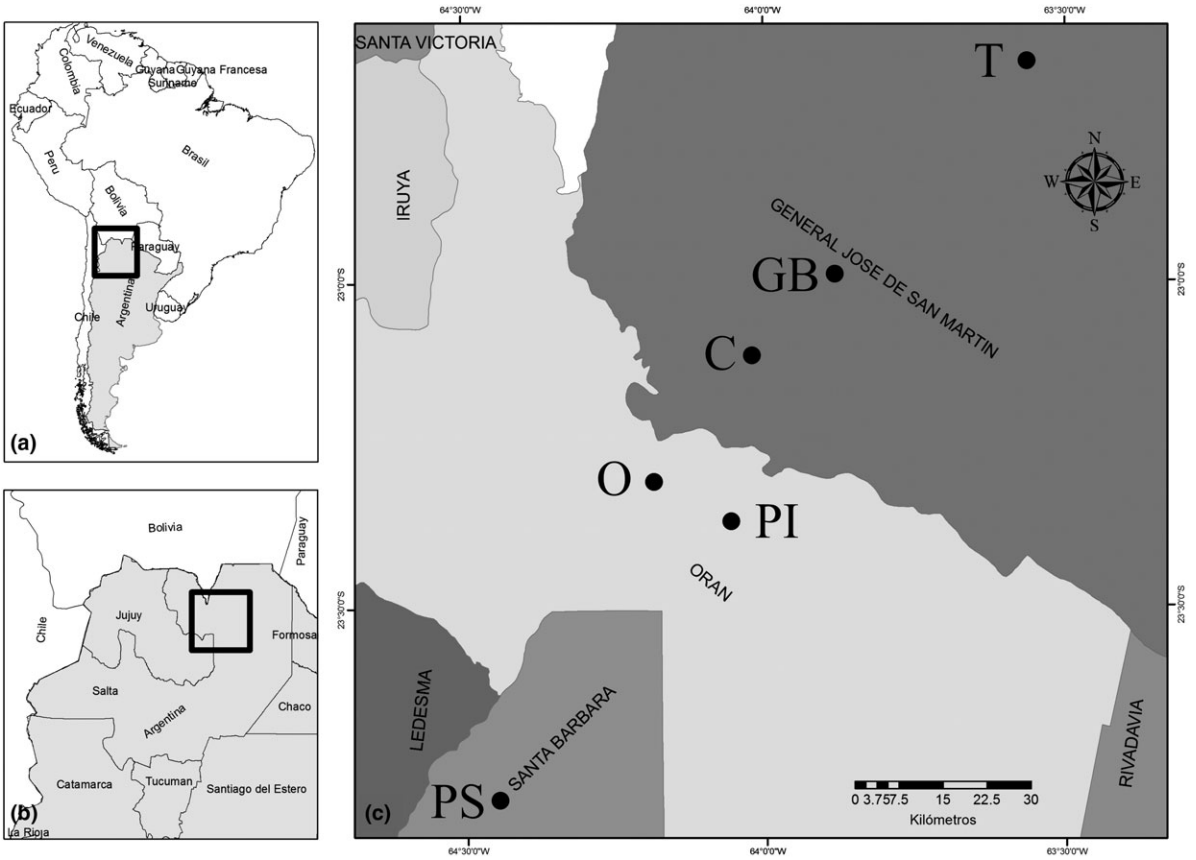

Figure 1 Map showing the locations of *Sclerotinia sclerotiorum* collection sites in northwestern Argentina. (a) Map of South America highlighting the location of northwestern Argentina; (b) map of northwestern Argentina showing the provinces sampled; (c) circles represent the location of the sampled sites: PS (Palma Sola), O (Orán), PI (Pichanal), C (Campichuelo), GB (General Ballivián) and T (Tartagal).

which represents the diversity within each location (Shannon & Weaver, 1963), was calculated for each geographic location as follows: $h_0 = -\sum (p_i \ln p_i)$, where p_i was the frequency of the *i*th MCG. Frequency was defined as the ratio between the number of isolates belonging to the *i*th MCG and the number of isolates in the sample. Because sample sizes differed among locations, MCG diversity values were normalized by the maximum diversity in each population as follows: $H_0 = h_0/\ln k$, where k was the sample size. The total MCG diversity (H_{tot}) was partitioned into within and among population components (Goodwin *et al.*, 1992). The clonal fraction was calculated for each population as 1 − [(number of different genotypes)/(total number of isolates)] (Zhan *et al.*, 2003).

Molecular characterization

Genomic DNA was extracted from 250 mg of hyphal tissue using an SDS protocol. The rDNA-ITS region of *S. sclerotiorum* isolates was amplified using primers ITS1 (5′-CTTGGTCATTTAGAG GAAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) as described by White *et al.* (1990). PCR amplification was performed in a 50 μL final volume containing 12–15 ng of genomic DNA, 1× reaction buffer (100 mM Tris.HCl pH 9.0, 500 mM KCl, 1% Triton X-100), 0.1 μM of each primer (Genbiotech S.R.L.), 200 μM of each dNTP, 3 mM $MgCl_2$ and 1 U *Taq* DNA polymerase (Highway-Inbio). The DNA amplifications were performed using a Master Cycler gradient thermocycler (Eppendorf) programmed with an initial denaturing step at 94 °C for 1 min; 30 cycles at 94 °C for 15 s, 58 °C for 15 s and 72 °C for 15 s; and a final extension cycle at 72 °C for 7 min. A 10 μL aliquot of the PCR product was resolved by electrophoresis through 1.5% (w/v) agarose gels stained with GelRed (Biotium) at 90 V in 1× TBE buffer for 1 h at room temperature. The fragments were visualized under UV light. The size of the DNA fragments was estimated by comparison with a 100–1000 bp DNA ladder (Highway-Inbio). The fragments were purified and subjected to sequencing in both directions using primers ITS1 and ITS4 on the 3500xL Genetic Analyzer sequencer (Applied Biosystems) at the Biotechnology Institute of INTA (Castelar, Buenos Aires, Argentina). The nucleotide sequences were subjected to basic local alignment search tool (BLAST) analysis (https://blast.ncbi.nlm.nih.gov) to identify isolates. Sequences were submitted to GenBank at the National Center for Biotechnology Information (NCBI) and accession numbers were obtained (Table S1). The multiple sequences and pairwise alignment were made using CLUSTALW (Thompson *et al.*, 1994) and adjusted manually with BIOEDIT v. 7.0.5 (Hall, 1999).

DNA amplification by URP-PCR

DNA from the 116 isolates was amplified using 11 URP primers as described by Kang *et al.* (2002). The reactions were performed in a 25 μL volume containing 50–100 ng of template DNA, 1× reaction buffer (100 mM Tris.HCl pH 9.0, 500 mM

KCl, 1% Triton X-100), 0.1 μM of each primer (Genbiotech S.R.L.), 200 μM of each dNTP, 3 mM $MgCl_2$ and 1.25 U *Taq* DNA polymerase (Highway-Inbio). The amplification programme began with an incubation at 94 °C for 4 min; then 35 cycles at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min; and a final extension at 72 °C for 7 min. The products of the reaction were electrophoresed as described above. Each reaction was repeated at least twice.

A binary matrix was constructed based on the amplification of all the isolates obtained with the URP primers. The presence and absence of fragments were scored as 1 and 0, respectively. Pairwise comparisons were calculated using Dice's similarity coefficient (Sneath & Sokal, 1973). The similarity matrix was used for a principal coordinates analysis (PCoA) to resolve patterns of variation among and within locations using InfoStat statistical software (Di Rienzo *et al.*, 2014). All amplifications were repeated in separate experiments at least twice for each isolate.

An analysis of the molecular variance (AMOVA) was applied to measure the genetic structure based on the correlation between haplotypes. Permutation tests were used to estimate the significance of the variance components. Population comparison was estimated through pairwise F_{ST} using Arlequin v. 3.5.2.2. (Excoffier *et al.*, 2005). Pairwise gene flow (N_m) was estimated for all populations based on the F_{ST} values (McDermott & McDonald, 1993). Linkage disequilibrium as an indication of random mating was calculated with the index of association, I_A and its standardized version, $\bar{r}_d$ using 999 permutations (Agapow & Burt, 2001) after clone-correction using the poppr package for R v. 2.5.0 (Kamvar *et al.*, 2014). Correlation between MCG, URP and geographic distance matrices was performed with Mantel's (1967) test.

Pathogenicity assay

The virulence of isolates was determined using the modified straw test (Terán *et al.*, 2006). Three seeds of black bean cv. INTA Leales 24 were sown in 15 cm diameter pots and grown under greenhouse conditions at 25 ± 2 °C (day) and 18 ± 2 °C (night) with a 12 h photoperiod. Mycelial cultures were established from stored stock cultures as previously described. Approximately 30 days after planting, the main stem was cut at the fourth node leaving a 3 cm internode intact (Terán *et al.*, 2006). A 200 μL Eppendorf tip stacked with two plugs of fresh mycelia from a PDA culture of each isolate, 48 h old, was placed mycelial-side down on the cut stem. Plants inoculated with the pure PDA plug without pathogen served as controls. The Eppendorf tip was allowed to stay on the top of the inoculated cut stem until the plant died, matured, or the Eppendorf tip fell off. Inoculated plants were incubated for 8 days under high humidity (>80%) using humidifiers in a growth chamber. White mould disease severity was evaluated by measuring lesion length (cm) on the main stem. A randomized complete design with three replicates (pots) consisting of three plants per pot was used. Analysis of variance was performed and means were compared using the DGC test ($P < 0.05$) in InfoStat statistical software (Di Rienzo *et al.*, 2014).

Results

A total of 116 isolates were collected from different bean fields in Salta and Jujuy provinces, northwestern Argentina (Table S1). Among the 116 isolates, the mycelium colour observed was white (71.6%), beige (25%) and brown (3.4%; Fig. S1). Different patterns of sclerotia formation were observed in the culture plates (Fig. S1). Fifty-four percent of the isolates showed a peripheral sclerotia pattern, whereas 27.6% showed central–peripheral sclerotia and 18.1% produced a central sclerotia pattern (Table S1). Significant differences were found in the average number of sclerotia per plate among all isolates, between and within the different localities ($P < 0.001$).

Mycelial compatibility groups

Fifty-two MCGs were identified among the 116 isolates of *S. sclerotiorum*. All isolates were self-compatible as shown by a continuous mycelium between paired colonies. The number of isolates within each MCG ranged from 1 to 18. Nineteen of the MCGs identified consisted of two or more isolates, and included 83 (72%) of the isolates analysed, while the remaining 33 MCGs were unique, which describes an MCG represented by a single isolate observed at a single location (Fig. 2a). The clonal fraction, which describes the proportion of isolates originating from asexual reproduction, was 55.2%.

An important mycelial incompatibility between fields was observed because all the observed MCGs were intransitive (no MCGs were shared between the evaluated fields). In addition, a high incompatibility within fields was observed, with a range of 3–20 MCGs per field, except for Orán that had mostly all compatible isolates (Table 1).

Shannon diversity index (H_0) of MCGs for all the samples analysed was 0.248 (H_{tot}), and ranged from 0.100 for Campichuelo to 0.301 for Tartagal (Table 1), with a mean of 0.196 (H_{pop}). Partition of total diversity (H_{tot}) showed that 79.2% corresponded to a variation of diversity within *S. sclerotiorum* populations, while only 20.8% of diversity was responsible for variability among those populations. The most diverse locations were General Ballivián and Tartagal.

Molecular characterization

Amplification of the ITS region yielded a single DNA fragment of approximately 540 bp, with uniform size among all isolates tested. A group of 108 randomly chosen PCR products were sequenced, and the ITS sequences were deposited in GenBank (MG516601–MG516708). The isolates had 100% similarity compared to *S. sclerotiorum* control sequences available in NCBI (JN012606.1, EF091807.1, AB233346.1, DQ329537.1).

Of the 11 URP primers evaluated, only five (URP-2R, URP-6R, URP-9F, URP-17R, URP-38F) showed different levels of polymorphism, with a total of 13 polymorphic bands. Amplified DNA bands ranged from 290 to 2000 bp for each isolate. A PCoA was performed based on the URP data. The first two principal coordinates explained 38.8% of the accumulated variation (Fig. S2).

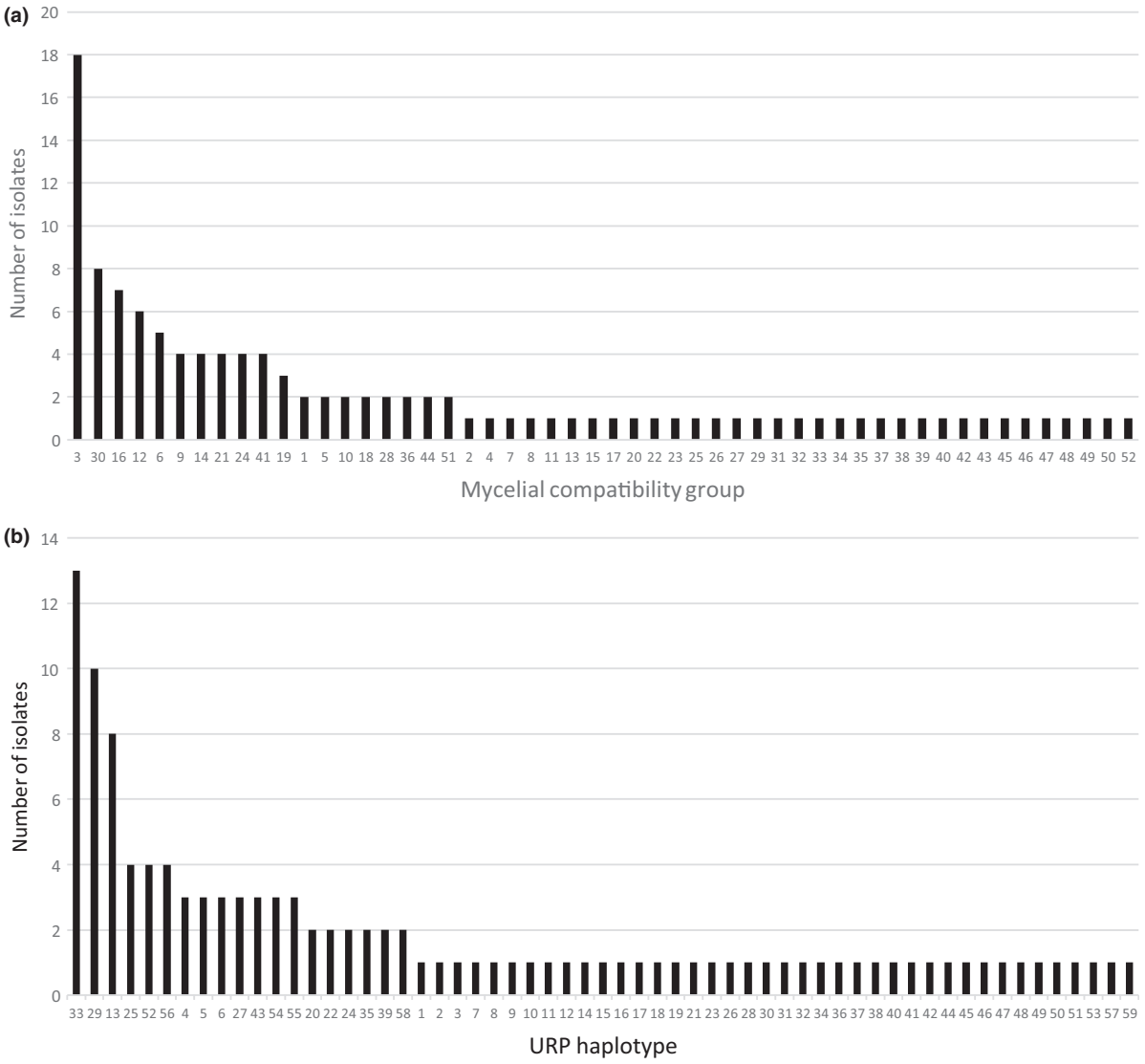

Figure 2 Histograms showing genotypic diversity among 116 isolates of *Sclerotinia sclerotiorum*. (a) Frequencies of 52 mycelial compatibility groups, (b) frequencies of 59 universal rice primer (URP) haplotypes.

There was a clear separation of isolates from General Ballivián and Tartagal on the first principal coordinate. Twenty-five (96%) isolates from General Ballivián tended to have more negative values along the first principal coordinate, while 31% (8) of the isolates from Tartagal showed high positive values. Also, most of the isolates from Orán (91%) showed more positive values on the first principal coordinate compared to the isolates from Campichuelo, Pichanal and Palma Sola.

Unique band patterns were obtained for 51% of the isolates, with a total of 59 haplotypes identified (Fig. 2b). Of the 59 haplotypes, 40 (68%) were unique, which describes a haplotype represented by a single isolate observed at a single location (Table S2). The remaining 19 haplotypes (32%) included 76 isolates (66%), seven haplotypes of which were found in at least two locations (Table S2). Haplotype 33 was the most abundant, represented by 13 isolates from two locations, General Ballivián (10) and Palma Sola (3). The clonal fraction was 49.14%.

Nearly all populations had a nonsignificant value of $\bar{r}_d$, which suggests that the null hypothesis of random mating cannot be rejected (Table S2). The exceptions were Tartagal and Orán which showed $\bar{r}_d$ values of 0.071 ($P < 0.001$) and 0.113 ($P < 0.01$), respectively. Both populations showed evidence of linkage, suggesting the existence of clonal reproduction or other forms of non-random mating.

The AMOVA showed a significant differentiation among populations (locations) ($\Phi_{ST} = 0.341$; $P < 0.0001$) with significant variation among and within populations (Table 2). Sixty-six percent of total genetic variation was

Table 1 Mycelial compatibility groups (MCGs) and MCG diversity (H_0) of *Sclerotinia sclerotiorum* populations from different sampling locations in Argentina.

Location	MCGs	Sample size	H_0[a]	H_{pop}[b]	H_{pop}/H_{tot}[c]	$(H_{tot} - H_{pop})/H_{tot}$[d]
Orán	1, 2, 3	21	0.131			
Pichanal	4, 5, 6, 7, 8, 9	14	0.168			
Campichuelo	10, 11, 12, 13, 14	14	0.100			
Palma Sola	15, 16, 17, 18, 19, 20	15	0.227			
General Ballivián	21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32	26	0.250			
Tartagal	33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52	26	0.301			
Total		116	0.248	0.196	0.792	0.208

[a]H_0, normalized MCG diversity.
[b]H_{pop}, the average H_0.
[c]H_{pop}/H_{tot}, the proportion of the total MCG diversity attributed to variations between individuals within a population.
[d]$(H_{tot} - H_{pop})/H_{tot}$, the proportion of total MCG diversity attributed to differences among populations.

Table 2 Analysis of molecular variance of *Sclerotinia sclerotiorum* isolates from six common bean fields in northwestern Argentina based on universal rice primer (URP) data.

Source of variation	d.f.[a]	SS[b]	MS[c]	Est. var.[d]	% of total variance[e]	Φ_{ST}	P[f]
Among population	5	75.639	15.128	0.721	34	0.341	<0.0001
Within population	110	153.439	1.395	1.395	66		
Total	115	229.078		2.116	100		

[a]Degrees of freedom.
[b]Sum of squares.
[c]Mean square.
[d]Estimate of variance.
[e]Percentage of total variance.
[f]*P*-value for randomization test for Φ_{ST} is based on 9999 permutations across the full data set.

associated with differences within populations, while 34% was due to the variation among populations (Table 2). Populations were genetically distinct based on pairwise F_{ST}, with values ranging from a low of 0.126 for a comparison of Campichuelo and Tartagal to a high of 0.557 for Orán and General Ballivián ($P \leq 0.0001$; Table 3). The only comparison that showed a nonsignificant F_{ST} value ($F_{ST} = 0.081$; $P = 0.058$) were Pichanal and Campichuelo populations. Correspondingly, the highest N_m values were observed between Pichanal and Campichuelo populations (Table 3). Gene flow was low (<1) between General Ballivián and all the other populations. Likewise, low values of N_m (<1) were observed between Orán and Pichanal, and Orán and Palma Sola (Table 3). In contrast, high N_m values (>1) were observed for the other comparisons, suggesting the existence of a moderate gene flow. These findings are in agreement with the results obtained with the PCoA.

The Mantel test was conducted to determine the relationship between genetic variation measured by the MCG and URP distance matrices. The correlation was significant, though of a low magnitude ($r = 0.25$, $P < 0.0001$). Comparison between both matrices and geographic distance between locations revealed a low correlation (Table S3).

Pathogenicity assay

As expected, all the isolates analysed were pathogenic to common bean (cv. INTA Leales 24) and produced typical white mould symptoms after 8 days of inoculation (Table S1). Lesion lengths differed significantly among isolates, ranging from 4.64 to 16.19 cm with a mean of 12.31 cm, resulting in the formation of two groups: weakly aggressive (WA) and highly aggressive (HA) isolates (Fig. S3). Most of the isolates tested were HA (93.8%) and were present in all the fields sampled. The seven WA isolates were found in four fields: General Ballivián (two), Palma Sola (three), Tartagal (one) and Pichanal (one). Only HA isolates were found on Orán and Campichuelo fields. Isolate SS116 (collected in General Ballivián) was the least aggressive (4.64 cm) of the WA group. On the other hand, isolate SS8 (collected in Orán) had the longest lesion length of 16.19 cm.

Discussion

In the present study, the diversity of *S. sclerotiorum* isolates from dry bean fields in the main production area of Argentina was characterized by means of molecular, morphological and pathogenic approaches. All the 116

Table 3 Population differentiation measured by F_{ST} (below diagonal) and gene flow (N_m) (above diagonal) between six *Sclerotinia sclerotiorum* populations in northwestern Argentina based on universal rice primer (URP) data.

Population[a]	O	PI	C	PS	GB	T
O	–	0.679	1.103	0.520	0.398	1.566
PI	0.424**	–	5.673	1.338	0.482	2.476
C	0.312**	0.081	–	2.217	0.649	3.468
PS	0.490**	0.272**	0.184**	–	0.696	1.674
GB	0.557**	0.509**	0.435**	0.418**	–	1.006
T	0.242**	0.168*	0.126*	0.230**	0.332**	–

Significance levels are based on *P*-values determined by 1023 permutations of the data. Values calculated were significantly different from zero at $P \leq 0.05$ (*) and $P \leq 0.0001$ (**). Gene flow was estimated as $N_m = (1 - F_{ST})/2F_{ST}$ (McDermott & McDonald, 1993).
[a]O, Orán; PI, Pichanal; C, Campichuelo; PS, Palma Sola; GB, General Ballivián; T, Tartagal.

isolates analysed were identified as *S. sclerotiorum* through ITS-rDNA sequence analysis and exhibited considerable genotypic and morphological variability.

Significant population differentiation by geographic location based on shared URP haplotypes and MCGs were found. A total of 52 MCGs and 59 molecular haplotypes were found, of which 33 (63%) and 40 (68%) were unique, respectively. All the MCGs were location-specific, while 12% of the URP haplotypes were shared among locations. Both techniques revealed higher genetic variability within the populations analysed than among them. These findings are in agreement with the intra- and interpopulation variability reported in Brazilian common bean fields (Gomes *et al.*, 2011), and in other crop fields in Argentina (Durman *et al.*, 2003), Iran (Karimi *et al.*, 2012) and Australia (Sexton & Howlett, 2004). Moreover, the F_{ST} values observed among locations indicates the existence of a significant population differentiation, with the exception of Pichanal and Campichuelo populations. General Ballivián was the most differentiated population, while Tartagal and Campichuelo were the least differentiated based on pairwise F_{ST} values. These observations were supported by the PCoA. Considering the shared haplotypes observed among populations and the N_m values estimated, the results suggest the existence of moderate gene flow between some locations. Migration of genotypes (exchange of haplotypes) among fields and sexual reproduction could be involved in maintaining the high level of MCG and haplotype variability observed. The existence of migration of genotypes among fields may occur through spreading fungal inocula in the form of sclerotia or mycelium by transporting contaminated soil in agricultural machinery (Abawi & Grogan, 1979). Considering that informal seed trade among local farmers is common in the region, pathogen dispersal by seeds contaminated with mycelium or sclerotia could have contributed to the introduction of new haplotypes from one field to another. In this region, the crop is cultivated without irrigation, so flowing water is discarded as a way of spreading inocula among fields. However, airborne ascospores, which constitute a common source of infection within fields, might be a mechanism for long-distance dispersal of haplotypes among populations (Abawi & Grogan, 1979). Moreover, in field observations carried out during the last growth cycle of the crop, it was possible to observe under suitable conditions of temperature and moisture, germinated sclerotia producing apothecia on the soil (M. Chocobar, unpublished observations).

Knowledge of genetic diversity and reproductive behaviour of the pathogen populations are important for breeding programmes because populations in which random mating takes place tend to evolve faster, raising the probability of overcoming host resistance or developing resistance to fungicides (McDonald & Linde, 2002). Several studies have characterized the genotypic diversity in *S. sclerotiorum* populations by means of MCG and fingerprinting to test for clonality (Carbone *et al.*, 1999; Sirjusingh & Kohn, 2001). Clonal populations are characterized by shared MCGs, with each MCG associated with only one molecular haplotype. On the other hand, in recombining populations, no association between MCG and fingerprint is observed (Cubeta *et al.*, 1997; Gomes *et al.*, 2011). There are studies on *S. sclerotiorum* population structure in canola, sunflower, cabbage and other crops fields reporting that populations are mainly clonal (Anderson & Kohn, 1995; Cubeta *et al.*, 1997). Also, the existence of both clonal and sexual reproduction in *S. sclerotiorum* populations in canola, cauliflower, carrot, sunflower, lettuce and pea has been reported (Malvárez *et al.*, 2007). In the present study, diversity among isolates collected in the same field was observed in MCGs and molecular haplotypes. Fourteen cases were considered clones, because two or more isolates belonged to the same MCG and shared the same URP haplotype. These cases were represented in all six locations studied. In contrast, there were another 14 cases in which one MCG was associated with more than one URP haplotype and 13 cases in which one URP haplotype was associated with more than one MCG, suggesting the occurrence of recombination. Clonal fractions of MCG and molecular haplotypes were 55.17% and 49.14%, respectively. Furthermore, evidence for outcrossing in most of the populations analysed was suggested by measures of multilocus linkage disequilibrium (I_A and $\bar{r}_d$). Thus, the results generated in this study suggest the occurrence of both clonal and sexual reproduction in *S. sclerotiorum* populations from common bean fields in northwestern Argentina. This is in agreement with the predominance of sexual reproduction in tropical

and subtropical populations of *S. sclerotiorum* (Cubeta *et al.*, 1997; Gomes *et al.*, 2011). However, a more thorough study, increasing the number of samples and analysing different agricultural zones using codominant markers as microsatellites, would be necessary to obtain comparable results.

Keeping seed to be used for the next crop is a common practice in the region which, combined with monoculture, favours the pathogen spread from one year to another by internally infected seed or sclerotia mixed with seed. A better understanding of the population dynamics of the pathogen over time will provide insight into the relative contribution of both modes of reproduction to genetic diversity in different locations and times, which is relevant for the selection of the most suitable breeding strategies for durable resistance.

The results also showed that the aggressiveness of *S. sclerotiorum* isolates was not related to their geographical origins, because most of the isolates were highly aggressive and isolates among locations did not vary widely in their level of aggressiveness. The lack of variation in aggressiveness among isolates from different geographic areas has been previously reported (Cubeta *et al.*, 1997; Durman *et al.*, 2003; Kull *et al.*, 2004; Sexton & Howlett, 2004; Malvárez *et al.*, 2007). No association between aggressiveness and MCGs and/or URP haplotypes was observed. Moreover, some weakly aggressive isolates had identical MCG and URP haplotypes to other highly aggressive isolates, for example SS174 and SS176, suggesting that these isolates have genetic differences that are not reflected by mycelial compatibility and URP markers.

The URP markers proved to be a useful technique for the population analysis of *S. sclerotiorum*, revealing high polymorphism among the isolates analysed. The results suggest that, despite their dominant nature, URP markers complemented with mycelial compatibility assays are a useful tool in providing preliminary information on the genetic structure and reproductive behaviour of *S. sclerotiorum*, as reported for other pathogens (González *et al.*, 2012; Mann *et al.*, 2014).

The information generated in the present study provides, for the first time, information on the variability and reproductive behaviour of *S. sclerotiorum* associated with white mould in the main common bean production areas in Argentina. These results may support the selection of representative local isolates for germplasm screening in regional common bean breeding programmes aimed to develop cultivars with durable resistance, and also for new fungicide screening. This work contributes to the development of sustainable management strategies such as plant resistance, biological and chemical control, and cultural practices in bean production aimed to minimize yield losses due to white mould.

Acknowledgements

C.L.A., G.T., Y.S. and M.A. are fellows of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. M.Z.G. and R.N.C. are CONICET researchers. This research was supported by INTA (PNHFA–1106075 and SALJU–1232307). The authors gratefully acknowledge Sergio Fontañez for his valuable contributions.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Variation in mycelium colour and different patterns of sclerotia distribution on potato dextrose agar plates of *Sclerotinia sclerotiorum* isolates after 15 days of incubation at 20 ± 2 °C. (a) White mycelium colour and central sclerotia pattern; (b) white mycelium colour and central-peripheral sclerotia pattern; (c) beige mycelium colour and peripheral sclerotia pattern; (d) brown mycelium colour and peripheral sclerotia pattern.

Figure S2. Bidimensional plot of principal coordinate analysis (PCoA) of 116 isolates of *Sclerotinia sclerotiorum* from northwestern Argentina using universal rice primer (URP) data.

Figure S3. Common bean plants (cv. Leales 24) inoculated with isolates of *Sclerotinia sclerotiorum* of different aggressiveness: (a) weakly aggressive, (b) highly aggressive, (c) control plant.

Table S1. Morphological characteristics, mycelial compatibility group (MCG), universal rice primer (URP) haplotype, lesion length, aggressiveness and GenBank accession number of 116 *Sclerotinia sclerotiorum* isolates collected from six common bean fields of Salta and Jujuy provinces in northwestern Argentina.

Table S2. Universal rice primer (URP) haplotype designations and frequencies associated with mycelial compatibility group (MCG) of *Sclerotinia sclerotiorum* isolates from common bean fields in northwestern Argentina.

Table S3. Correlation analysis among mycelial compatibility groups (MCGs), universal rice primer (URP) data and geographic distance between locations (*r*: lower diagonal; *P*-value: upper diagonal).