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ORIGINAL ARTICLE

Characterization of mating type and the diversity of pathotypes of *Phytophthora infestans* isolates from Southern Brazil

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Abstract
Late blight is caused by the oomycete *Phytophthora infestans*, and it is the most devastating disease of potato in Southern Brazil. The objective of this work was to evaluate the variability of *P. infestans* isolates collected from potatoes in Rio Grande do Sul (RS), Paraná (PR) and Santa Catarina (SC) states for their mating type (*MT*) and virulence. One hundred and twenty-eight isolates of *P. infestans* were collected between 2010 and 2012. They were characterized for mating type and subsequently evaluated for virulence/avirulence using a differential series of 11 potato clones. The virulence study was conducted in vitro by the leaf disc method, where the potato clones were inoculated with each isolate. Seventy-six isolates were identified as *MT*A2, 24 as *MT*A1, 17 as *MT*A1A2 and 11 as self-fertile. In addition, 79 pathotypes of *P. infestans* were detected in the survey and the largest number of races was identified in RS. The most frequent pathotypes identified were '14' and '21' and the vast majority of isolates overcame the R7 resistance gene, followed by the R3, R1 and R11 genes. Smaller numbers of virulent isolates were detected to the clones carrying the R5, R2 and R9 genes. Higher percentages of virulent and complex isolates were identified in the *MT*A2. High indices of diversity were observed in populations grouped by mating type and by state of collection. The highest value of the Gleason index $H_{GR} = (0.95)$ was obtained for the isolates from PR. Higher complex *P. infestans* populations were found in RS, and the least complex population was in PR.

Keywords *Solanum tuberosum* · Late blight · Mating type · Virulence · Races · Variability

Introduction

Late blight, caused by the oomycete *Phytophthora infestans*, is the most destructive disease of potato worldwide (Li et al. 2009; Fry et al. 2015) and has been responsible for costly sprays to potato growers (Rodewald and Trognitz 2013). The outbreaks of late blight on potato crops that occurred in Europe in the nineteenth century were associated with the HERB-1 strain of *P. infestans* which persisted for the next 50 years (Yoshida et al. 2013). This genotype is distinct from the US1 lineage that until recently was hypothesized to be responsible for the occurrence of the late blight epidemics in the nineteenth century (Goodwin et al. 1994). The US1 lineage belonging to A1 mating type replaced the HERB-1 lineage and was dominant until the early 1980s in different parts of the world (Yoshida et al. 2013). In fact, the A2 mating type was restricted to Mexico until by the end of the 1970s (Spielman et al. 1991). Since then, the distribution of *P. infestans* mating types in the world has changed over the years (Fry 2008; Cooke et al. 2012; Ibrahin and Taleb-Hossenkhan 2017; Rekad et al. 2017).

Genetic changes in the population of *P. infestans* in a given region occur primarily by migration of exotic strains with specific genotypic and epidemiological characteristics (Cooke et al. 2012; Fry et al. 2015). According to Fry (2008), the structure of the global population of *P. infestans* began to change during the late twentieth

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century. One cause of this change was the migration of isolates of the A2 mating type from Mexico to the USA and Europe. The first record of this occurred in the European continent during the early 1980s (Hohl and Iselin 1984). Since then, the A2 mating type group has been found in other countries, including Brazil, where the clonal lineage of the A2 group was named BR-1 (Goodwin and Drenth 1997), and whose prevalence has been reported in potato crops by Reis et al. (2003). Recent surveys showed that the *MT*A2 is still predominant on potato in Brazil, but the *MT*A1 has also been found in potato crops in Rio Grande do Sul, São Paulo and Minas Gerais states (Oliveira 2010; Santana et al. 2013).

Migration has been the main phenomenon responsible for the emergence of new *P. infestans* strains from both mating types with higher aggressiveness and virulence (Spielman et al. 1991; Sjöholm et al. 2013; Saville et al. 2016) The coexistence of populations of both A1 and A2 mating types in the same area would increase the probability of emergence of new genotypes (Widmark et al. 2011; Cooke et al. 2012).

In studies of characterization of populations of *P. infestans*, phenotypic characteristics such as virulence of the pathogen on a series of differential potato clones containing single R genes for qualitative resistance are often investigated due to the frequent high variability of physiological races of the pathogen (Malcolmson and Black 1966; Guo et al. 2009; Sedlák et al. 2017).

The resistance R genes were identified in the wild species *Solanum demissum*, and a differential set of R genes (R1–R11) has been developed. This differential series has been used for the identification of the races of *P. infestans* (Malcolmson and Black 1966; Harbaoui et al. 2013). During the past century, these R genes were transferred to *S. tuberosum*, creating high expectations for the control of *P. infestans*. However, this resistance was not durable because it is race specific (Fry 2008) and the pathogen is highly variable. The R genes encode receptors that recognize the secretion of effectors such as proteins (*Avr*) produced by *P. infestans*. These proteins induce resistance in hosts containing the R gene; the reverse occurs on a host that does not have a resistance gene (Akino et al. 2014). However, these resistance genes can be overcome by different races of *P. infestans* (Fry 2008).

In many parts of the world, there is a wide variability of physiological races of *P. infestans* on potato crops, going from simple to the most complex races (Malcolmson 1969; Li et al. 2009; Delgado et al. 2013; Harbaoui et al. 2013; Sedlák et al. 2017). Studies conducted by Reis (2001) and Santana (2006) in Brazil have also shown a great variability within of pathotypes of this pathogen. However, Reis did not use all differential series and Santana tested a small number of pathogen isolates. According to Samen et al. (2003), the genetic diversity of races is calculated using diversity indices for verifying the existence of differences in levels of diversity within and between populations of *P. infestans*.

The aim of this study was to evaluate the variability and complexity of isolates of *P. infestans* from potato growing in Rio Grande do Sul, Paraná and Santa Catarina states for mating type and virulence to a differential series of potato clones containing single resistance genes, as well as to determine the genetic diversity of pathogen pathotypes.

Materials and methods

Sampling and isolation

Leaf and stem samples of potato plants infected with *P. infestans* were collected from conventional, organic and trial fields in the major potato-producing regions during the growing season in the states of Rio Grande do Sul, Santa Catarina and Paraná in the south of Brazil during the period from 2010 to 2012. The samples were collected in eighty-eight fields. The majority of these fields were conventional varying from two to twenty sprays of fungicides per growing season (Table 1). One hundred and twenty-eight isolates of *P. infestans* were isolated as follows: pieces of leaflet and stem excised from the edges of single lesions were incubated at 18 °C in darkness for 24 h in plastic boxes (Gerbox) with one sheet of filter paper moistened with sterilized distilled water until the emergence of the sporangia (until sporulation). Monosporangial isolates of *P. infestans* were obtained from the samples onto rye A agar medium as described in Caten and Jinks (1968) and maintained at 18 °C for ten or twelve days. Grown colonies were purified and maintained in agar slants covered by mineral oil for 1 to 6 months till they were used in virulence tests. Before the virulence test, all isolates were recovered, inoculated on the cultivar Craigs Royal and reisolated from this cultivar.

Phenotypic analyses

Mating type

Mating type was determined by pairing each isolate with standards A1 (US-1) or A2 (BR-1) isolates (Santana et al. 2013), and self-paired on plates containing clarified V8 juice agar and incubated at 18 °C in the dark. After 18 days of incubation, each Petri dish was observed using a microscope to monitor oospore formation. When oospores were found in the pairing with the A2 tester, the isolate was classified as belonging to the A1 mating type and vice versa. Isolate-producing oospores with both standard isolates were designated A1A2 group. Finally, isolates that produced oospores with both standard isolates and also alone were considered as self-fertile genotypes.

Table 1 Origin and number of fields sampled with *Phytophthora* infestans in the southern states of Brazil: Parana (PR), Santa Catarina (SC) and Rio Grande do Sul (RS), in the years 2010–2012 and number of sprays of fungicides

Isolates	Municipality	State[a]	Year collected	Latitude	Longitude	Field[b]	Samples	Field system	Sprays of fungicide
1-CANG	Canguçu	RS	2010	−31°17′07″	−52°36′58″	1	1	C	2
2CANG	Canguçu	RS	2010	−31°07′33″	−52°28′08″	2	1	C	DNC[c]
2 NP	Nova Petrópolis	RS	2010	−29°23′05″	−51°01′58″	3	1	C	3
3NP	Nova Petrópolis	RS	2010	−29°24′21″	−51°03′20″	4	1	C	4
4-1 NP	Nova Petrópolis	RS	2010	−29°23′37″	−51°03′38″	5	5a	C	4
4-2 NP	Nova Petrópolis	RS	2010	−29°23′37″	−51°03′38″	5	5b	C	4
5NP	Nova Petrópolis	RS	2010	−29°12′47″	−51°03′38″	6	1	C	3
10MR	Morro Reuter	RS	2010	−29°32′20″	−51°03′14″	7	1	C	3
11MR	Morro Reuter	RS	2010	−29°33′08″	−51°03′01″	8	1	C	4
8 SMH	S. M. do Herval	RS	2010	−29°29′00″	−51°00′44″	9	1	C	5
2-SvM	Silveira Martins	RS	2010	−29°37′51″	−53°35′38″	10	1	C	2
3 SvM	Silveira Martins	RS	2010	−29°37′42″	−53°34′04″	11	1	C	5
4-SvM	Silveira Martins	RS	2010	−29°39′11″	−53°33′58″	12	1	C	5
5SvM	Silveira Martins	RS	2010	−29°38′51″	−53°32′54″	13	1	C	8
6-SvM	Silveira Martins	RS	2010	−29°38′33″	−53°33′00″	14	1	C	4
9-SvM	Silveira Martins	RS	2010	−29°44′54″	−53°34′39″	15	1	C	6
Pel-2 a	Pelotas	RS	2011	−31°36′24″	−52°31′48″	16	16a	C	4
Pel-2b	Pelotas	RS	2011	−31°36′51″	−52°31′49″	16	16b	C	4
Pel-2e	Pelotas	RS	2011	−31°36′27″	−52°31′50″	16	16c	C	4
Pel-2f	Pelotas	RS	2011	−31°36′25″	−52°31′49″	16	16d	C	4
Pel-03	Pelotas	RS	2011	−31°36′16″	−52°31′40″	17	1	C	DNC
Pel-05	Pelotas	RS	2012	DNC	DNC	18	1	O	DNC
Pel-06	Pelotas	RS	2012	−31°40′20″	52°26′14″	19	1	T	No spray
Pel-07	Pelotas	RS	2012	DNC	DNC	20	1	C	2
Pel-08	Pelotas	RS	2012	DNC	DNC	21	1	T	2
Campo-2	Pelotas	RS	2011	−31°41′10″	−52°26′11″	22	1	T	No spray
Emb-C	Pelotas	RS	2011	−31°40′30″	−52°26′06″	23	1	T	No spray
2 CES	Pelotas	RS	2012	DNC		24	1	DNC	DNC
CRI-1a	Cristal	RS	2011	-31°02′11″	−52°06′16″	25	1	C	7
CRIS-02	Cristal	RS	2011	−31°02′15″	−52°06′17″	26	26a	C	6
CRI-2b	Cristal	RS	2011	−31°02′16″	−52°06′18″	26	26b	C	6
CRI-03	Cristal	RS	2011	−31°02′24″	−52°06′54″	27	1	C	7
CRI-4 a	Cristal	RS	2011	−31°02′35″	−52°06′51″	28	28a	C	7
CRI-4b	Cristal	RS	2011	−31°02′44″	−52°06′50″	28	28b	C	7
CRI-5a	Cristal	RS	2011	−31°02′54″	−52°06′49″	28	28c	C	7
CRI-5b	Cristal	RS	2011	−31°02′62″	−52°06′47″	28	28d	C	7
CRI-6	Cristal	RS	2011	−31°03′37″	-52°07′57″	29	29 a	C	7
CRI-7 a	Cristal	RS	2011	−31°03′30″	−52°07′52″	29	29b	C	7
CRI-08-a	Cristal	RS	2011	−31°05′18″	−52°07′39″	30	30a	C	6
CRI-8b	Cristal	RS	2011	−31°05′24″	−52°07′46″	30	30b	C	6
CRI-9	Cristal	RS	2011	−31°05′30″	−52°07′50″	30	30c	C	6
CRI-10	Cristal	RS	2011	−31°04′24″	−52°04′45″	31	1	C	7
SL-01a	S. L. do Sul	RS	2011	−31°12′44″	−51°56′48″	32	32a	C	6
SL-01b	S. L. do Sul	RS	2011	−31°12′38″	−51°56′40″	32	32b	C	6
SL-4b	S. L. do Sul	RS	2011	−31°10′56″	−52°02′40″	33	33a	C	5
SL-4d	S. L. do Sul	RS	2011	−31°10″59″	−52°02′43″	33	33b	C	5
SL-5b	S. L. do Sul	RS	2011	−31°13′05″	−53°05′28″	34	1	C	7
CRIS	S. L. do Sul	RS	2010	DNC	DNC	35	1	O	No spray
IBI-1b	Ibiraiaras	RS	2011	−28°23′04″	−51°35′16″	36	36a	C	6

Table 1 (continued)

Isolates	Municipality	State[a]	Year collected	Latitude	Longitude	Field[b]	Samples	Field system	Sprays of fungicide
IBI-1d	Ibiraiaras	RS	2011	−28°23′01″	−51°35′14″	36	36b	C	6
IBI-01e	Ibiraiaras	RS	2011	−28°23′03″	−51°35′12″	36	36c	C	6
IBI-1f	Ibiraiaras	RS	2011	−28°23′02″	−51°35′10″	36	36d	C	6
IBI-2b	Ibiraiaras	RS	2011	−28°23′10″	−51°35′08″	37	1	C	6
IBI-3-a	Ibiraiaras	RS	2011	−28°22′54″	−51°36′47″	38	1	C	5
IBI-4b	Ibiraiaras	RS	2011	−28°22′56″	−51°36′43″	39	1	DNC	DNC
IBI-05	Ibiraiaras	RS	2011	−28°22′59″	−51°36′33″	40	1	DNC	DNC
IBI-06 a	Ibiraiaras	RS	2011	−28°22′02″	−51°44′34″	41	41a	C	6
IBI-6 b	Ibiraiaras	RS	2011	−28°21′58″	−51°44′30″	41	41b	C	6
IBI-6e	Ibiraiaras	RS	2011	−28°21′59″	−51°44′36″	41	41c	C	6
3SJA	S. J. dos A.	RS	2012	−28°44′20″	−50°01′40″	42	1	C	2
SJA-6	S. J. dos A.	RS	2012	−28°43′19″	−50°07′56″	43	43a	C	8
SJA-7	S. J. dos A.	RS	2012	−28°43′21″	−50°07′54″	43	43b	C	8
SJA-8	S. J. dos A.	RS	2012	−28°43′16″	−50°07′50″	44	1	C	10
SJA-9	S. J. dos A.	RS	2012	−28°44′20″	−50°05′48″	45	1	C	5
SJA-10	S. J. dos A.	RS	2012	−28°36′59″	−49°55′11″	46	46a	C	6
SJA-12	S. J. dos A.	RS	2012	−28°36′57″	−49°55′13″	46	46b	C	6
SJA-13	S. J. dos A.	RS	2012	−28°36′50″	−49°55′06″	46	46c	C	6
SJA-14	S. J. dos A.	RS	2012	−28°38′25″	−49°56′32″	47	47a	C	8
SJA-15	S. J. dos A.	RS	2012	−28°38′28″	−49°56′28″	47	47b	C	8
SJA-16	S. J. dos A.	RS	2012	−28°38′20″	−49°56′37″	48	1	C	8
SJA-18	S. J. dos A.	RS	2012	−28°38′26″	−49°56′18″	49	1	C	6
BJ-01	Bom Jesus	RS	2012	−28°37′15″	−50°25′04″	50	50a	C	5
BJ-02	Bom Jesus	RS	2012	−28°37′18″	−50°25′06″	50	50b	C	5
BJ-03	Bom Jesus	RS	2012	−28°37′19″	−50°25′09″	50	50c	C	5
BJ-04	Bom Jesus	RS	2012	−28°37′16″	−50°25′08″	50	50d	C	5
BJ-05	Bom Jesus	RS	2012	−28°37′07″	−50°25′00″	51	1	C	5
BJ-07	Bom Jesus	RS	2012	−28°36′55″	−50°29′41″	52	1	C	8
BJ-08	Bom Jesus	RS	2012	−28°37′04″	−50°29′28″	53	1	C	8
SFP-01	S. F. de Paula	RS	2012	−29°22′43″	−50°25′14″	54	54a	C	9
SFP-2	S. F. de Paula	RS	2012	−29°22′32″	−50°25′20″	54	54b	C	9
SFP-3	S. F. de Paula	RS	2012	−29°22′31″	−50°25′30″	55	1	C	9
SFP-6	S. F. de Paula	RS	2012	−29°27′40″	−50°20′31″	56	56a	C	5
SFP-7	S. F. de Paula	RS	2012	−29°27′37″	−50°20′25″	56	56b	C	5
SFP-08	S. F. de Paula	RS	2012	−29°27′39″	−50°20′26″	56	56c	C	5
RG-01	Rio Grande	RS	2012	DNC	DNC	57	1	DNC	DNC
MF-01	Mafra	SC	2012	−26°10′01″	−49°51′06″	58	58a	C	4
MF-03	Mafra	SC	2012	−26°10′02″	−49°51′07″	58	58b	C	4
MF-04	Mafra	SC	2012	−26°10′04″	−49°51′08″	58	58c	C	4
MF-05	Mafra	SC	2012	−26°10′06″	−49°51′11″	58	58d	C	4
MF-06	Mafra	SC	2012	−26°10′09″	−49°51′01″	59	1	DNC	DNC
SC-08	Canoinhas	SC	2011	−26°11′24″	−50°21′58″	60	1	T	No spray
SC-11	Itaiópolis	SC	2011	−26°24′18″	−49°49′27″	61	61a	C	9
SC-12	Itaiópolis	SC	2011	−26°24′12″	−49°49′19″	61	61b	C	9
SC-12-1	Itaiópolis	SC	2011	−26°24′02″	−49°49′08″	61	61c	C	9
SC-15	Três Barras	SC	2011	−26°17′37″	−50°07′02″	62	1	C	20
SC-16	São Joaquim	SC	2011	−28°16′33″	−49°56′40″	63	1	T	No spray
SJ6a1	São Joaquim	SC	2011	−28°16′59″	−49°55′23″	64	64a	O	DNC
SJ-6b1	São Joaquim	SC	2011	−28°16′52″	−49°55′20″	64	64b	O	DNC
SCSJ7	São Joaquim	SC	2011	−28°16′55″	−49°18′35″	65	1	C	2

Table 1 (continued)

Isolates	Municipality	State[a]	Year collected	Latitude	Longitude	Field[b]	Samples	Field system	Sprays of fungicide
SC-C3	Canoinhas	SC	2012	−26°05′08″	−50°23′03″	66	1	S.i	DNC
SC-C4	Canoinhas	SC	2012	−26°04′46″	−50°23′50″	67	1	C	4
SC-C7	Canoinhas	SC	2012	−26°05′17″	−50°32′24″	68	1	C	6
SC-C9	Canoinhas	SC	2012	26°11′23″	−50°21′48″	69	1	T	No spray
SC-C11	Major Viera	SC	2012	−26°21′31″	−50°20′48″	70	70a	C	5
SC-C12	Major Viera	SC	2012	−26°21′21″	−50°20′41″	70	70b	C	5
SC-3	S.M. do Sul	PR	2011	−26°01′10″	−50°34′51″	71	1	DNC	DNC
PR-01 a	Palmas	PR	2011	−26°29′22″	−52°03′19″	72	1	DNC	DNC
PR-14	Araucária	PR	2011	−25°42′22″	−49°23′56″	73	1	C	10
PR-18	Araucária	PR	2011	−25°44′09″	−49°23′41″	74	1	C	4
PR-20	Araucária	PR	2011	−25°42′25″	−49°23′43″	75	1	O	14
PR-24	Contenda	PR	2011	−25°40′31″	−49°29′49″	76	1	C	6
PR-25	Lapa	PR	2011	−25°46′51″	−49°45′42″	77	77a	T	8
PR-26	Lapa	PR	2011	−25°46′51″	−49°45′42″	77	77b	T	8
PRGU1	Guarapuava	PR	2011	−25°20′39″	−51°40′22″	78	1	C	15
PRGU3	Guarapuava	PR	2011	−25°13′43″	−51°44′52″	79	1	C	15
PRGU-5	Guarapuava	PR	2011	−25°12′36″	−51°41′59″	80	1	C	15
AR-02	Araucária	PR	2012	−25°44′16″	−49°23′37″	81	1	C	3
PR-PL3a	Palmas	PR	2011	−26°33′56″	−51°46′29″	82	82a	C	12
PRPL-3b	Palmas	PR	2011	−26°42′25″	−51°23′43″	82	82b	C	12
PR-3b	Pinhão	PR	2011	−25°48′16″	−51°43′57″	83	1	C	14
PGR-1	Ponta Grossa	PR	2012	−25°12′28″	−50°07′12″	84	84a	C	16
PGR-2	Ponta Grossa	PR	2012	−25°12′24″	−50°07′06″	84	84b	C	16
PGR-4	Ponta Grossa	PR	2012	−25°12′18″	−50°07′02″	84	84c	C	16
CLAR-2	Campo Largo	PR	2012	−25°23′40″	−49°29′17″	85	1	DNC	DNC
CON-1	Contenda	PR	2012	−25°38′49″	−49°34′24″	86	86a	C	3
CON-02	Contenda	PR	2012	−25°38′52″	−49°34′26″	86	86b	C	3
CON-8	Contenda	PR	2012	−25°38′59″	−49°33′31″	87	1	C	4
BN-2	Balsa Nova	PR	2012	−25°33′31″	−49°36′27″	88	1	C	10

[a]State of South of Brazil: RS = Rio Grande do Sul, SC = Santa Catarina, PR = Paraná

[b]Number of fields of potato where isolates were collected

[c]DNC Data not collected

Virulence assays

The isolates were evaluated for virulence to a series of eleven clones of potato, each clone containing a single R gene for qualitative resistance (R1–R11) derived from *Solanum demissum* (Malcolmson and Black 1966; Harbaoui et al. 2013). As control, the cultivar Craig's Royal (R0), without any vertical resistance genes, was used (Solano et al. 2016).

The virulence assay was carried out on leaf discs of each clone (Hermansen et al. 2000). Forty days after planting, the leaves of each genotype were collected for immediate use in the experiment. The side leaflets were used for obtaining leaf discs, and the apical leaflet was discarded. Leaf discs measuring 15 mm in diameter were cut out from the leaves with the aid of a cork borer. Discs were arranged on filter paper moistened with sterile water, abaxial surface up within a Petri dish.

For obtaining pathogen inoculum, the isolates of *P. infestans* were grown on rye B Agar medium for 10 to 12 days (Caten and Jinks 1968). Subsequently, a liquid suspension of each isolate was prepared containing 10^5 sporangia per ml. Thereupon, each isolate was inoculated on the abaxial surface of the leaf discs of each series of differentiating clones, using a 20 µL volume of the suspension per disc according to the methodology of Sozzi et al. (1992). The experiment was conducted in a completely randomized design with three replications. One Petri dish, containing five leaf discs, was considered a repetition. After inoculation, the Petri dishes were kept in an incubation chamber at 18 ± 1 °C with a photoperiod of 16 h light for 6 days. When

necessary, sterilized water was added to the plate to prevent drying of the filter paper.

The virulence of isolates of *P. infestans* was evaluated with a stereomicroscope based on a scale of disease severity notes (SD), according to the methodology of Sozzi et al. (1992), which ranged from 0 to 5, where: 0 = no symptoms; 1 = leaf necrosis; 2, 3, 4 and 5 account for 5%, 5 to 20%, 20 to 50% and > 50% of the leaf disc surface covered by sporulation of the oomycete, respectively. From these data, it was determined the reaction of virulence compatibility and incompatibility between each *P. infestans* isolate and every one of the potato clones with a single resistance R gene. When the sporulation of the oomycete was clearly visible on the leaf disc, starting with note two, the reaction was considered compatible and when it was not possible to be clearly observed (score < 2), it was classified as incompatible according to the methodology described previously (Santana 2006; Delgado et al. 2013). If the result was not conclusive, the isolate was tested again.

Pathotype diversity and complexity

The pathotype (race) diversity within *P. infestans* populations was calculated through the relative Gleason index, $H_{GR} = N_p - 1/N_i - 1$, where N_p is the number of different races and N_i is the number of isolates evaluated. This index is derived from the Gleason index (Andrivon and Vallavieille-Pope 1993). The relative Gleason index was calculated to reduce the bias resulting from comparisons between populations with different sample sizes since this index varies with the sample size (Andrivon 1994).

Additionally, two indices of virulence complexity were calculated: $C_i = \Sigma\ (p_i v_i)$ (the mean number of virulence genes per isolate) and $C_p = (1/n)\ \Sigma v_i$ (the mean number of virulence alleles per pathotype—each identified pathotype is considered only once), where p_i and v_i are the frequency and the number of virulence alleles, respectively, of the *i*th pathotype in the sample, and *n* is the number of different pathotypes in the sample (Andrivon and De Vallavieille-Pope 1993).

Results

Mating type and virulence assays

Out of 128 isolates evaluated, 76 were characterized as belonging to mating type A2, including 52 isolates from Rio Grande do Sul (RS) state, 12 isolates from Santa Catarina (SC) state and 12 isolates from Parana (PR) state. Twenty-four isolates were characterized as A1 group with 17 isolates from RS, four isolates from SC and three isolates from PR states. Seventeen isolates were scored as A1A2 including 16 isolates from RS and one isolate from SC state, and 11 were scored as self-fertile with three isolates from SC and eight isolates from PR states (Tables 2 and 4).

Seventy-nine physiological races of *P. infestans* were identified. The most complex race identified was '01', represented by only the one isolate (1.3% of isolates), containing 11 virulence (1.2.3.4.5.6.7.8.9.10.11) genes with occurrence only in RS state. However, the most common pathotypes were '21' (1.3.4.7.8.10.11) (10.1%) and '14' (1.3.4.6.7.8.10.11) (7.6%), present in the three states and containing seven and eight virulence genes, respectively. Moreover, considering all detected pathotypes, 81.0% were virulent to more than four clones (Table 2).

The largest number of pathotypes identified was found in samples collected in Rio Grande do Sul ($n = 61$), followed by the states of Paraná ($n = 22$) and Santa Catarina ($n = 16$). Analyzing the pathotypes for mating type, 54 physiological races of *P. infestans* were found in the group A2, 21 races in the group A1, 17 in the A1A2 group and ten in the population of the self-fertile isolates. Among the 76 isolates of *MT*A2, 61.8% were virulent to more than six R genes; in the *MT*A1 group, there was 58.3% of 24 isolates; in the A1A2 group 47.0% of the 17 isolates and 18.8% of the 11 self-fertile isolates also were virulent to more than six R genes (Tables 2 and 4).

When evaluating the product of the *Avr* gene of *P. infestans* isolates, recognized by the R genes of different potato clones, two isolates (SJA-12 and PR-01a) expressed all the avirulence genes. Three isolates (Pel-2e, BJ-04 and SC-08) had 10 *Avr* genes. Ten isolates had nine *Avr* genes, nine isolates eight *Avr* genes and 103 isolates presented one to seven avirulence genes. In the evaluation of races, 78 out of 79 pathotypes expressed one to eleven *Avr* genes and only one isolate (IBI-4b) presented no avirulence gene. When the frequency of the avirulence genes was evaluated, the *Avr*7, *Avr*3, *Avr*1, *Avr*11 and *Avr*4 were less frequent. Otherwise, the *Avr* genes *Avr*5, *Avr*2 and *Avr*9 were the most frequent (Table 2).

Pathotype diversity and complexity

Among the isolates from Southern Brazil, the intraspecific diversity of pathotypes, given by the relative Gleason index (H_{GR}), was 0.61. However, when it was calculated by state, the highest level of genetic diversity occurred in Paraná, whose index was 0.95, followed by the states of Santa Catarina ($H_{GR} = 0.79$) and Rio Grande do Sul ($H_{GR} = 0.71$). The genetic diversity of pathotypes of *P. infestans* on the basis of mating type showed that the A1A2 group had the highest index of diversity ($H_{GR} = 1.00$), followed by self-fertile isolates with $H_{GR} = 0.90$, *MT*A1 with $H_{GR} = 0.87$ and *MT*A2 with $H_{GR} = 0.71$ (Table 4).

Table 2 Mating types, races and virulence/avirulence genes of *Phytophthora* infestans isolates from the Southern Region of Brazil

Pathotype	Virulence/avirulence	Isolates	Place[a]			MT[b]
			RS	SC	PR	
01	0.1.2.3.4.5.6.7.8.9.10.11	IBI-4b	1	–	–	A1
02	0.1.2.3.4.6.7.8.9.10.11/*5*	Pel-03, CRI-2b, CRI-10	3	–	-	A1A2-A1-A2
03	0.1.2.3.4.5.6.7.8.10.11/*9*	BJ-05	1	–	–	A2
04	0.1.3.4.5.6.7.8.9.10.11/*2*	Pel-2b	1	–	–	A1A2
05	0.1.2.3.4.5.6.7.8.9.11/*10*	Campo-2	1	–	–	A2
06	0.1.2.3.4.6.7.9.10.11/*5.8*	IBI-1b, CRI-1a, CRI-08-a	3	–	–	A2-A1A2-A2
07	0.1.3.4.5.6.7.8.10.11/*2.9*	MF-03	–	1	–	A2
08	0.1.2.3.5.6.7.9.10.11/*4.8*	CRI-6	1	–	–	A2
09	0.1.2.3.4.5.6.7.9.11/*8.10*	IBI-06a	1	–	–	A2
10	0.1.2.3.4.6.7.8.9.11/*5.10*	IBI-3-a	1	–	–	A2
11	0.1.2.3.4.6.9.10.11/*5.7.8*	CRI-7a	1	–	–	A1
12	0.1.2.3.6.7.9.10.11/*4.5.8*	1-CANG	1	–	–	A2
13	0.1.2.4.6.7.9.10.11/*3.5.8*	CRI-8b	1	–	–	A1A2
14	0.1.3.4.6.7.8.10.11/*2.5.9*	IBI-6b, SJA-15, SJA-7, SC-12, AR-02, MF-05	3	2	1	A1-A1-A2-A2-A2-SF
15	0.1.3.4.5.7.8.10.11/*2.6.9*	IBI-2b, BJ-08, BJ-01	3	–	–	A1-A2-A2
16	0.1.3.4.5.6.7.9.10/*2.8.11*	SFP-08	1	–	–	A1A2
17	0.1.2.3.4.6.7.9.11/*5.8.10*	SL-01b, SL-01a, CRI-4a	3	–	–	A2-A1A2-A2
18	0.1.2.3.4.6.7.8.9/*5.10.11*	SC-C9	–	1	–	A1
19	0.1.2.3.7.8.9.11/*4.5.6.10*	2-SvM	1	–	–	A2
20	0.1.3.4.6.7.10.11/*2.5.8.9*	IBI-01e, PR-PL3a, PRPL-3b	1	–	2	A2-A1-A2
21	0.1.3.4.7.8.10.11/*2.5.6.9*	SJA-13, SJA-8, SFP-3, MF-01, SCSJ7, PRGU1, SJA-18, IBI-1f	5	2	1	A1-A1-A2-A2-A1-A2-A2-A2
22	0.2.3.6.7.9.10.11/*1.4.5.8*	CRI-5b	1	–	–	A2
23	0.2.3.4.6.7.9.11/*1.5.8.10*	SFP-01	1	–	–	A2
24	0.2.3.4.7.8.9.11/*1.5.6.10*	SJA-10	1	–	–	A1
25	0.1.2.3.4.6.7.10/*5.8.9.11*	6-SvM	1	–	–	A2
26	0.1.3.4.6.7.9.10/*2.5.8.11*	Pel-06	1	–	–	A1A2
27	0.2.3.4.6.7.8.9/*1.5.10.11*	IBI-05	1	–	–	A2
28	0.1.2.3.4.6.7.9/*5.8.10.11*	CRI-03, SL-4d, Emb-C, 3NP	4	–	–	A2-A1A2-A2-A2
29	0.1.2.4.7.9.11/*3.5.6.8.10*	9-SvM	1	–	–	A2
30	0.1.3.7.8.10.11/*2.4.5.6.9*	BJ-02, SC-C11	1	1	–	A2-A2
31	0.1.3.4.7.10.11/*2.5.6.8.9*	5NP, SC-C12, SC-C7, SJA-6	2	2	–	A2-A2-A2-A2
32	0.1.3.4.8.10.11/*2.5.6.7.9*	SC-11	–	1	–	A2
33	0.3.4.7.8.10.11/*1.2.5.6.9*	MF-04	–	1	–	A2
34	0.1.4.7.8.10.11/*2.3.5.6.9*	PGR-1			1	SF
35	0.1.3.6.7.8.11/*2.4.5.9.10*	SJA-9	1	–	–	A1
36	0.1.3.4.7.8.11/*2.5.6.9.10*	SJA-16, SFP-6, IBI-1d, 2CANG, PR-20	4	–	1	A2-A2-A2-A1A2-A2
37	0.1.3.4.7.9.11/*2.5.6.8.10*	RG-01	1			A2
38	0.1.3.4.7.8.10/*2.5.6.9.11*	PR-14	–	–	1	A1
39	0.1.2.3.4.5.7/*6.8.9.10.11*	11MR	1	–	–	A2
40	0.1.3.7.10.11/*2.4.5.6.8.9*	CLAR-2	–	–	1	A2
41	0.1.3.7.8.11/*2.4.5.6.9.10*	BJ-07, SJ6a1, SC-C4	1	2	–	A2, A1A2, SF
42	0.1.3.4.7.11/*2.5.6.8.9.10*	SC-15, PR-25	–	1	1	A2-A2
43	0.3.4.7.10.11/*1.2.5.6.8.9*	SJ-6b1, 10MR	1	1	–	A1, A1A2
44	0.2.3.7.9.11/*1.4.5.6.8.10*	CRI-4b	1	–	–	A2
45	0.3.6.7.9.10/*1.2.4.5.8.11*	Pel-08	1	–	–	A1
46	0.3.6.7.9.11/*1.2.4.5.8.10*	5SvM	1	–	–	A2
47	0.3.4.7.8.11/*1.2.5.6.9.10*	4-2 NP	1	–	–	A2

Table 2 (continued)

Pathotype	Virulence/avirulence	Isolates	Place[a]			MT[b]
			RS	SC	PR	
48	0.1.4.7.8.10/*2.3.5,6.9.11*	SC-12-1	–	1	–	A2
49	0.1.2.3.6.7/*4.5.8.9.10.11*	SL-4b	1	–	–	A2
50	0.1.3.4.7.9/*2.5.6.8.10.11*	Pel-2a	1	–	–	A1A2
51	0.1.3.4.7.8/*2.5.6.9.10.11*	IBI-6e, SC-C3	1	1	–	A2-A2
52	0.2.4.6.9.11/*1.3.5.7.8.10*	SL-5b	1	–	–	A1A2
53	0.4.7.10.11/*1.2.3.5.6.8.9*	PR-18		–	1	A2
54	0.2.3.7.10/*1.4.5.6.8.9.11*	Pel-07	1	–	-	A1A2
55	0.1.3.7.10/*2.4.5.6.8.9.11*	PR-24	-	–	1	A2
56	0.1.3.7.11/*2.4.5.6.8.9.10*	2 CES, CON-8	1	–	1	A2, SF
57	0.2.3.7.11/*1.4.5.6.8.9.10*	SFP-7	1	–	–	A2
58	0.1.6.7.11/*2.3.4.5.8.9.10*	4-SvM	1	–	–	A2
59	0.2.7.9.11/*1.3.4.5.6.8.10*	8 SMH	1	–	–	A2
60	0.1.7.8.11/*2.3.4.5.6.9.10*	PRGU-5	–	–	1	A2
61	0.1.2.3.7/*4.5.6.8.9.10.11*	2 NP	1	–	–	A1
62	0.1.3.4.7/*2.5.6.8.9.10.11*	4-1 NP	1	–	–	A2
63	0.1.3.7.9/*2.4.5.6.8.10.11*	Pel-05, CRI-5 a	2	–	–	A2-A1
64	0.3.4.7.8/*1.2.5.6.9.10.11*	SC-3	–	–	1	A1
65	0.1.7.11/*2.3.4.5.6.8.9.10*	SC-16, PR-3b	–	1	1	A1-A2
66	0.1.4.11/*2.3.5.6.7.8.9.10*	CRIS, PGR-4	1	–	1	A2-SF
67	0.3.7.11/*1.2.4.5.6.8.9.10*	3 SvM, CRI-9	2	–	–	A1-A2
68	0.2.3.7/*1.4.5.6.8.9.10.11*	Pel-2f	1	–	–	A1A2
69	0.3.4.7/*1.2.5.6.8.9.10.11*	SJA-14, PR-26	1	–	1	A1-A2
70	0.1.11/*2.3.4.5.6.7.8.9.10*	CRIS-02, BN-2	1	–	1	A1- SF
71	0.8.11/*1.2.3.4.5.6.7.9.10*	CON-02	–	–	1	SF
72	0.7.11/*1.2.3.4.5.6.8.9.10*	3SJA, PRGU3	1	–	1	A2-A2
73	0.3.11/*1.2.4.5.6.7.8.9.10*	CON-1, MF-06	–	1	1	SF-SF
74	0.3.7/*1.2.4.5.6.8.9.10.11*	SFP-2	1	–	–	A1A2
75	0.4.7/*1.2.3.5.6.8.9.10.11*	BJ-03	1	–	–	A2
76	0.1.8/*2.3.4.5.6.7.9.10.11*	PGR-2	–	–	1	SF
77	0.4/*1.2.3.5.6.7.8.9.10.11*	SC-08	–	1	–	A2
78	0.7/*1.2.3.4.5.6.8.9.10.11*	Pel-2e, BJ-04	2	–	–	A1A2-A2
79	0/*1.2.3.4.5.6.7.8.9.10.11*	SJA-12, PR-01a	1	–	1	A1-SF
Total of isolates by state			85	20	23	128
Total of pathotypes						79

[a]State where isolates were collected: RS = Rio Grande do Sul, SC = Santa Catarina, PR = Paraná

[b]MT = mating type; SF = self-fertile

Pathotype complexity varied from one virulence gene (overcoming only the clone with no R gene) in two isolates from Rio Grande do Sul and Paraná (mating types A1 and SF) to 12 virulence genes detected in one isolate from Rio Grande do Sul belonging to the A1 mating type (Table 2). There was apparently no relationship among pathotype complexity, based on C_i and C_p indices, with pathotype diversity, based in the H_{GR} index (Table 4).

The subpopulation from Rio Grande do Sul was the most complex ($C_i = 9.96$ and $C_p = 6.87$) followed by the subpopulation from Santa Catarina ($C_i = 8.13$ and $C_p = 6.44$), and the subpopulation from Paraná state was the least complex ($C_i = 5.70$ and $C_p = 5.05$). The subpopulations from the A1, A2 and A1A2 mating types presented similar complexity, but the self-fertile subpopulation presented a much lower complexity than the others (Table 4).

Discussion

The occurrence of mating types A1, A2 and variations A1A2 and self-fertile isolates in the South of Brazil is probably a consequence of migration. Brazilian potato growers cultivate lots of potato seeds imported from Europe and North America where all mating types are present in potato fields (Fry 2008; Fry et al. 2015). Populations of *P. infestans* worldwide have changed mainly because of gene flow (Saville et al. 2016). Additionally, sexual reproduction generates new genotypes. However, the existence of two mating type in one region is not sufficient to conclude the appearance of a sexual population (Fry et al. 2015). The introduction of a new mating type in a region can cause changes in the population structure of *P. infestans,* in some cases with the generation of new virulence factors (Drenth et al. 1994), and the appearance of new pathogen genotypes that are more aggressive. As an example, the lineage 13_A2 replaced the old lineages in Great Britain and overcame the resistance of the local cultivars (Cooke et al. 2012).

The self-fertility that occurs in *P. infestans* is also termed secondary homotalism (Fry et al. 2015). This kind of isolates has already been reported in many parts of the world (Tantius et al. 1986; Han et al. 2013; Orona et al. 2013) and recently in Brazil (Casa-Coila et al. 2017). In the last years, self-fertile genotypes of *P. infestans* have been dominant in some regions of China. However, the genetic, evolutionary and ecological causes of the occurrence of these pathogen variants are not yet known (Zhu et al. 2015, 2016). Evidently, the occurrence of self-fertile genotypes affects the genotypic diversity in a population of *P. infestans* (Han et al. 2013). The mating type A1A2 has been also uncommonly reported (Statsyuk et al. 2010; Li et al. 2009). In Brazil, A1A2 isolates were previously reported in the Rio Grande do Sul state (Santana 2006; Santana et al. 2013). In this study, this uncommon mating type was present mostly in Rio Grande do Sul. These isolates differ from autofertiles because of their inability to form oospores when submitted by themselves on plates containing clarified V8 juice agar.

The mating type A2 isolates presented complex pathotypes. In a work carried out by Santana (2006), most isolates carrying more than five R genes belonged to mating type A2. In Tunisia, isolates from the A2 group showed more complex races than the group A1, and the A2 isolates were also more aggressive and tolerant to high temperatures (Harbaoui et al. 2013). However, in studies conducted by Knapova and Gisi (2002), most isolates belonging to the A1 group were virulent to more than six qualitative R genes. Other studies conducted in Ecuador showed that the population of *P. infestans* with the A1 mating type was virulent to four to eleven R genes (Delgado et al. 2013). In Brazil, the low frequency of complex pathotypes in the A1 group was previously reported by Santana (2006). The smaller number of complex pathotypes in the A1 group can be explained by the recent re-emergence of this group on potatoes in Brazil, since previous studies have reported that the *P. infestans* population was mostly from the A2 mating type (Reis et al. 2003). Thus, the results obtained in this study and those described in several other studies show that the occurrence of complex isolates is not a specific characteristic of a particular mating type.

The large number ($n = 79$) and high complexity of *P.infestans* races found in this study, with a ratio of one pathotype to 1.62 isolates, is indicative of the high variability in the population of *P. infestans* in the south region of Brazil. Although Reis (2001) and Santana (2006) have observed a high diversity of pathotypes of *P. infestans* in isolates collected in potato and tomato fields from the south and southeast regions of Brazil, the number of pathotypes observed in this study was at least twice the number found by those two authors. The two complex races '14' and '21', observed as the most frequent in the three states, have also been reported previously as the most frequent in potato production areas of Switzerland and France (Knapova and Gisi 2002).

The highest frequency of virulent isolates was on the clones containing the R7, R3, R1, R11 and R4 genes, and it is lower in clones with R5 genes, R2 and R9 (Table 2) coinciding with the reactions found by Li et al. (2009). The exception was the clone with the R1 gene, which showed a low frequency of susceptibility in the work performed by Guo et al. (2009). Knapova and Gisi (2002) also observed a high frequency of virulence on the clones R7, R3, R1, R11 and R4. However, in this work there was no compatible interaction between the pathogen isolates and the clones containing the R5 and R9 genes. Comparing the reactions found in this study with those obtained by Reis (2001), in Brazil, the reactions of clones with the R5 and R2 genes were similar. However, it was not possible to compare with R9 because this gene was not tested in that work. In relation to the studies of Santana (2006), the frequency of pathotypes against the R2 gene in Southern Brazil increased from 15% to 35.4%, and on the clone containing the gene R9 it increased from 0.0 to 38%. However, in relation to the R5 gene, the virulence frequency decreased from 18% to 12.6%. Although in the past the strategy of using R genes has not had lasting success, more recently the combination of qualitative and quantitative resistance has been one of the main objectives in potato breeding programs aimed at achieving greater durability of resistance over the years (Fry 2008; Stewart et al. 2003).

The high pathotype diversity found within populations of *P. infestans* from the southern region of Brazil and the occurrence of complex pathotypes can be a consequence

Table 3 Number and percentage of *Phytophthora* infestans isolates virulent on eleven race differential clones of potato carrying different R genes for resistance to late blight

R gene	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11
Number of virulent isolates ($n=128$)[a]	94	39	103	84	12	46	114	53	40	56	93
Percentage (%)	73.4	30.5	80.5	65.6	9.3	35.9	89.0	41.4	31.2	43.7	72.7

[a]Number of isolates virulent on each potato clone carrying one R gene

Table 4 Genetic diversity and complexity of races of populations of *P. infestans* from the southern region of Brazil ordered by origin and mating type

State	N_i[a]	N_p[b]	H_{GR}[c]	C_i[d]	C_p[e]
RS[f]	85	61	0.71	9.96	6.87
SC[g]	20	16	0.79	8.13	6.44
PR[h]	23	22	0.95	5.70	5.05
Southern Region	128	79	0.61	10.89	6.57
MT[i]	N_i	N_p	H_{GR}	C_i	C_p
A1	24	21	0.87	7.57	6.80
A2	76	54	0.71	9.36	6.74
A1A2	17	17	1.00	7.37	7.58
SF[j]	11	10	0.90	4.70	4.40

[a]*N*i = number of isolates

[b]*N*p = number of pathotypes

[c]H_{GR} = Relative Gleason index

[d]*C*i = the mean number of virulence genes per isolate

[e]C_p = the mean number of virulence alleles per pathotypes

[f]RS = Rio Grande do Sul

[g]SC = Santa Catarina

[h]PR = Paraná

[i]MT = mating type

[j]SF = self-fertile

of the occurrence of both mating types and/or migrations from other countries. According to Drenth et al. (1994) in the Netherlands until 1980, only the *MT*A1 was present and after the appearance of *MT*A2 new virulence factors were found, increasing enormously the diversity of virulence of isolates of *P. infestans*. Consequently, there was genetic diversity increase. Thus, the occurrence of *MT*A1 and *MT*A2 in potato crops in Southern Brazil can be among the mechanisms that influence the phenotypic and genotypic diversity of *P. infestans* because of the occurrence of sexual reproduction, which provides new pathogen genotypes with specific and unexpected characteristics (Fry et al. 2015), despite there is no proof of sexual reproduction of the pathogen in Brazil. Other mechanisms such as parasexual events, mutation, gene and genotype flow, migration, random genetic drift and selection carried out by the host also allow the occurrence of pathogen variability (McDonald and Linde 2002; Knapova and Gisi 2002; Li et al. 2009).

Despite the higher number of pathotypes of *P. infestans* found in Rio Grande do Sul state (Table 3), a higher diversity index was observed in the population of isolates collected in Paraná, which indicates an increase in the pathotype diversity in this state as compared to that obtained by Santana (2006), fewer than 10 years ago. The greatest pathotype diversity found in the *P. infestans* population from Paraná State can be due to the existence of self-fertile isolates that can be generating progenies with new virulence characteristics (Table 4).

Even though potato cultivars cultivated in Southern Brazil carry few or no R genes, most isolates collected in this Brazilian region belonged to complex races. Other authors have also found high levels of virulence in *P. infestans* populations that cannot be explained by the *R* gene constitution of the potato cultivars (Malcolmson 1969; Hermansen et al. 2000). Thus, some authors argued that the concept of "stabilizing selection" proposed by Van der Plank (1968) is not important in the *P. infestans* × potato pathosystem (Tooley et al. 1986; Forbes et al. 1997).

Differences in pathotype diversity between these three contiguous states may be largely driven by genetic drift and low migration rates. Host selection pressure is ruled out because almost the same potato cultivars are grown in the three states of Southern Brazil. Mutation would not be solely responsible for the differentiation, considering the mutation rate being similar regardless of geographic region. Genetic drift and migration are likely to be involved in the differentiation process. Random genetic drift, a process that becomes increasingly important as population size decreases, can lead to different allele frequencies or the fixation of distinct alleles in isolated populations through the random sampling of genes over generations (McDermott and McDonald 1993).

Considering the high diversity within populations of *P. infestans* in Southern Brazil and its distribution in the growing areas, strategies based on the use of R genes are not likely to be successful. Hence, the tomato and potato breeding programs in Brazil must look for quantitative resistance to late blight. The temporal dynamics of the avirulence genes in the field is also an important fact to consider in breeding programs. Periodic assessment of pathotype diversity must be conducted. A new survey must start to assess diversity no more than a decade after the present study. The identification and knowledge of the distribution of pathotypes of *P. infestans* are essential for planning control measure

strategies. Among these control strategies, researchers must focus on the development of potato genotypes with durable resistance to the pathogen.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals Not applicable.

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