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Article

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Genetic variability of *Rhizoctonia solani* Kühn and its resistance to fungicides

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Abstract

The aims of this study are to determine the population genetic structure of *Rhizoctonia solani*, associated with potato, and to assess its resistance degree to pencycuron and thiabendazole fungicides. A total of 57 strains of *R. Solani* were isolated from different potato crop fields in Mexico, and were classified according to their anastomosis group (AG) using the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP)-based analysis. Then, the amplified fragments were digested with four different endonucleases (*Mse* I, *Ava* II, *Mun* I and *Hinc* II). The Repetitive Element Sequence-Based PCR (rep-PCR) technique was used to determine the genetic diversity in *R. solani* populations. Results obtained by PCR-RFLP showed that 81% of the isolates were identified as belonging to AG-3 group, 14% to AG1-1B and 5% to AG-11 group. In response to fungicides inhibition, IC_{50} between 0.014-0.039 mg a.i./L for pencycuron, and 0.82 to 2.91 for thiabendazole were determined, This IC_{50} value showed that the resistance factor (RF) values ranged from 1.4 to 3.945 and 0.27 to 0.97, respectively; this tendency suggests that isolates are susceptible to thiabendazole. The AG groups showed a heterogeneous resistance to pencycuron and thiabendazole, being more tolerant to these fungicides AG1-1B and AG-11 groups. The genetic analysis shows a low genetic diversity among (P-value 0.3225) and within (P-value 0.3275) populations; this can indicate a clonal reproduction and little parasexual cycle among the analyzed isolations.

Key words – Anastomosis group – genetic-diversity – genetic-resistance – Mexico – PCR-RFLP – parasexual cycle – REP-PCR – Wright's statistics

Introduction

In Mexico, potato is one of the most important annual crops and because of the total area dedicated to this commodity and its high production costs, most of the crop cost is spent on pest control. *Rhizoctonia solani* [*Teleomorph*: *Thanatephorus cucumeris* (*Frank*) *Donk*] is a pathogenic fungus that attacks potato (*Solanum tuberosum* L.), where the main observed symptoms in damaged plants are black scurf on tubers and stem; and stolon canker on underground stems. For

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this reason, the disease is called black scurf disease of potato. This disease occurs wherever potatoes are grown, however, *R. solani* causes economically significant damage when growing climatic conditions are cool, and the soil is wet (Banville 1989). However, indistinctly from the producing area, pencycuron and thiabendazole fungicide applications are carried out to avoid infection, since the production of black-spotted tuber loses quality and commercial value. The frequent application of fungicide can induce resistance in plant pathogens (Chen et al. 2012, Mu et al. 2017).

Usually, the AG-3 group is the most commonly associated with potato production areas (Ceresini et al. 2002, Woodhall et al. 2007, Lehtonen et al. 2008a, Bolton et al. 2010), although another AG are reported such as: AG-2, AG-4, AG-5 and AG-7 (Holguin 1999, Virgen et al. 2000, Hernández et al. 2001, 2005, Woodhall et al. 2007). *Rhizoctonia solani* is commonly found in soil, infecting more than 200 plant species (Ogoshi 1996, Lehtonen et al. 2008a, Wibberg et al. 2013, Hane et al. 2014, Hannukkala et al. 2016, Huynh & Akihiro 2016). Based on hyphal anastomosis between strains, 14 genetically distinct groups (AG) had been defined (Carling et al. 2002, González 2002, Guillemaut et al. 2003, Wibberg et al. 2013, Hane et al. 2014). Each AG is considered an independent entity, evolutionary or phylo-species (Cubeta & Vilgalys 1997, Carling et al. 2002, Pannecoucque et al. 2008, Sturrock et al. 2015) because exchange, and recombination occurs only between compatible strains (Anderson 1982, Stacy & Kenneth 2004). The specific identification of the anastomosis group is related to efficient disease management, because each anastomosis group has differential pathogenicity, and response to temperature, soil texture, etc. (Pannecoucque et al. 2008, Bolton et al. 2010, Goswami et al. 2011), and sensitivity to fungicides (Kataria et al. 1991, Lehtonen et al. 2008b, LaMondia 2012).

The knowledge of *R. solani* in regard to amount and distribution of genetic variation within and between groups is important for understanding biology of pathogen populations, and to infer the relative impact of different evolutionary forces that influence its biology, and thus predict the evolutionary potential of populations within agricultural ecosystems. The studies which generate information contribute to a better understanding of crop-pathogen patho-system, and therefore aid to design new management strategies through knowledge of their genetic structure, and monitoring of fungicides sensitivity. The objectives of this study were: 1) to determine the genetic variability of *R. solani* anastomosis groups more commonly found in different Mexican potato (*Solanum tuberosum*) regions, and 2) to estimate *R. solani* AG resistance to pencycuron and thiabendazole fungicides.

Materials & Methods

Sampling locations

In randomly chosen commercial lots from five Mexican states (Nuevo Leon, Coahuila, Sonora, Jalisco and Chihuahua) potato tubers, and tissues were collected. Each sample was identified, and transferred under controlled temperature conditions (5 ± 1°C) to the Agricultural Parasitology Department the Universidad Autonoma Agraria Antonio Narro in Saltillo, Mexico.

Isolation of *Rhizoctonia solani* strains

Strains of *Rhizoctonia solani* were isolated from plant tissue with disease symptoms, and sclerotic tissue present in tubers. Fragments adjacent to wound and/or sclerotic tissue were cut, and disinfected with sodium hypochlorite at 3% (v/v), for two minutes, then, rinsed three times with sterile distilled water. After, fragments were allowed to dry and seeded on potato dextrose-agar (PDA). The fragments were incubated at 24 ± 0.2°C, until fungal tissue was observed. The colonies were purified though the hyphal tip from colonies, with macroscopic characteristics typical of *R. solani*, such as development, and change of mycelial color, and appearance; branching of hyphae, and existence of multinucleate cells were also determined (Parmeter & Whitney 1970).

Anastomosis group characterization

The AG identification of each strain was performed using the PCR-RFLP technique (Guillemaut et al. 2003). Total genomic DNA was isolated from fresh mycelium (100 mg) by Dellaporta et al. (1983) technique, after which DNA integrity was analyzed by electrophoresis in agarose gels (1%); staining with ethidium bromide and visualizing with UV light. This DNA was amplified by PCR using 25 µL final volume amplification reaction: DNA100 ng (2 µL), buffer 10x (2.5 µL), $MgCl_2$ 50 mM (1 µL), dntp's 2mM (2 µL), primer 10 pM (1.5 µL), and Taq DNA polymerase at 5U/µL (0.25 µL). The PCR program: 94°C for 5 min, 35 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min (Guillemaut et al. 2003).

Each PCR product was digested using the endonucleases *Mse* I, *Ava* II, *Mun* I and *Hin*c II, separately; and after this, sample was incubated at 37°C for 3 hours. These enzymes are specific to differentiate among *R. solani* anastomosis groups. Ten µL of the PCR product was electrophoresed on 1.5% agarose gel, stained with ethidium bromide, illuminated and documented using Biorad UV Transilluminator. Each digestion enzyme generated a pattern from fungal DNA, which was encoded and compared with the specific code obtained for each reference strain (Guillemaut et al. 2003).

Genetic Variability of *Rhizoctonia solani*

Amplification of fungal DNA by repetitive extragenic palindromic elements (REP) from each *R. solani* strain was performing by PCR using ERIC1 primer (Godoy et al. 2004). The reaction was composed as follows: 2 µL of 100 ng DNA, 2.5 µL of 10x buffer, 1 µL of 50 mM MgCl2, 2 µl of DNTP's at 2 mM, 1.5 µL of 10 pM primer, and 0.25 µL of 5U/µL Taq DNA polymerase. PCR program was as follow: an initial denaturation for 7 min at 95°C, 30 cycles of 1 min at 94°C, 1 min at 53°C and 8 min at 72°C and a final extension of 10 min at 72°C.Ten µL of the PCR product were electrophoresed on 1% agarose gel, stained with ethidium bromide, illuminated and documented using Biorad UV Transilluminator. The polymorphism pattern was coded using a binary system (1 = presence, 0 = absence); this data matrix was analyzed employing the info- Gen software (Balzarini & Di-Rienzo 2003) to determine genetic diversity and analysis of molecular variance.

Resistance of *Rhizoctonia solani* anastomosis groups to fungicides

Each *R. solani* strain was grown on culture medium containing pencycuron (0, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75 and 1.0 ppm) and thiabendazole (0, 0.5, 1.0, 1.5, 2.0, 3.0 and 5.00 ppm). On this medium, a mycelia disc (0.5 cm) with active growth of each *R. solani* strain was placed, then the Petri Dish was sealed and incubated at 25 ± 1°C. The radial growth was measured until the fungal strain growth in culture media without fungicide, completely covered the Petri dish. Each value was converted to inhibition percent of radial growth and this mycelial inhibition percent was employed to perform Probit analysis by maximum likelihood method using the SAS software (V.8.0) (http://support.sas.com/documentation/onlinedoc/v8/whatsnew/). The estimate doses on mycelial inhibition (%) were obtained at a probability of 95%. Later, the 50% (IC_{50}) and 90% (IC_{90}) inhibitory concentrations of each strain were obtained. The resistance factor (RF) was estimated by dividing the IC_{50} value of each strain by the IC_{50} value of the most susceptible reported strain. The RF indicates the number of times that an individual is more tolerant to an active substance than a susceptible individual (Leroux 1987).

Results

Morphological characteristics

A total of 57 *Rhizoctonia* spp. isolates were obtained from samples from potato plant roots, and tall with typical symptoms of Rhizoctonia root rot was isolated, as well as tuber sclerotic tissue.

Anastomosis group characterization

Restriction analysis of PCR products with enzymes, revealed different patterns. When these

were compared to the patterns of the reference strains reported, it was possible to identify each strain at the level of anastomosis group (Fig. 1).

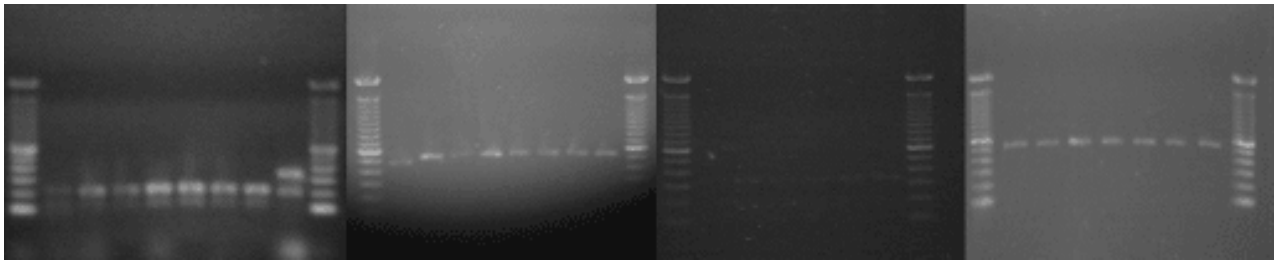

Fig. 1 – Restriction analysis generated by *Mse* I, *Ava* II, *Hin*c II and *Mun* I, enzymes (Sonora strains).

In Table 1, it can be observed that the AG-3 anastomosis group was found at the highest frequency and represented 81 % of all fungal isolates, followed by AG1-1B with 14%, and finally the AG-11 with 5%.

Table 1 *Rhizoctonia solani* anastomosis group obtained by restriction profile comparative.

Strain	**Origen**	**Anastomosis group**	**Strain**	**Origen**	**Anastomosis group**
SO1	Sonora	AG11	A72	Collection	AG3-PT
SO2	Sonora	AG3-PT	H33	Collection	AG3-PT
SO3	Sonora	AG3-PT	IS	Collection	AG3-PT
SO4	Sonora	AG3-PT	A26	Collection	AG11
SO5	Sonora	AG3-PT	A77	Collection	AG3-PT
SO6	Sonora	AG3-PT	A31	Collection	AG3-PT
SO8	Sonora	AG3-PT	A79	Collection	AG3-PT
SO9	Sonora	AG3-PT	JCH51	Chihuahua	AG3-PT
PC4	Coahuila	AG3-PT	NCCH1	Chihuahua	AG3-PT
SJVC1	Coahuila	AG3-PT	NCCH2	Chihuahua	AG3-PT
SJVC2	Coahuila	AG3-PT	NCCH3	Chihuahua	AG3-PT
SJVC3	Coahuila	AG3-PT	NCCH4	Chihuahua	AG3-PT
HC1	Coahuila	AG1-1B	NCCH5	Chihuahua	AG3-PT
PC2	Coahuila	AG3-PT	NCCH6	Chihuahua	AG3-PT
HC2	Coahuila	AG3-PT	CA1	Canada	AG3-PT
PC3	Coahuila	AG3-PT	CA2	Canada	AG11
SJVC4	Coahuila	AG1-1B	CA4	Canada	AG3-PT
SRNL1	Nuevo Leon	AG1-1B	CA7	Canada	AG1-1B
SRNL2	Nuevo Leon	AG3-PT	CA8	Canada	AG3-PT
SJNL1	Nuevo Leon	AG3-PT	CA9	Canada	AG3-PT
SJNL2	Nuevo Leon	AG3-PT	76106	Reference	AG8
SJNL4	Nuevo Leon	AG3-PT	76129	Reference	AG6
SJNL5	Nuevo Leon	AG3-PT			
PNL1	Nuevo Leon	AG3-PT			
PNL2	Nuevo Leon	AG1-1B			
TJ1.1	Jalisco	AG3-PT			
TJ1.2	Jalisco	AG3-PT			
TJ1.3	Jalisco	AG3-PT			
TJ2.1	Jalisco	AG3-PT			
TJ2.2	Jalisco	AG3-PT			
TJ3.1	Jalisco	AG3-PT			
TJ3.3	Jalisco	AG3-PT			
TJ4.1	Jalisco	AG3-PT			
TJ4.3	Jalisco	AG3-PT			
TJ5.1	Jalisco	AG1-1B			

Anastomosis groups of *Rhizoctonia solani* and its resistance to fungicides

The Probit analysis showed IC_{50} values indicating that resistance of pencycuron fungicide ranges from 0.014 to 0.039 (Table 2). There are reports that IC_{50} for a *R. solani* strain is 0.01 mg a.i. /L (Kataria et al. 1989). With these data, it was determined that the resistance factor (RF) of the studied strains ranged from 1.4 to 3.945.

Table 2 Inhibitory concentration at 50 and 90% (IC_{50} and IC_{90}), and resistance factors to pencycuron fungicide in *Rhizoctonia solani* strains from different Mexican potato regions.

Strains	IC_{50} (mg/L)	LFL	UFL	IC_{90} (mg/L)	RF	AG
CA1	0.014	0.011	0.018	0.66	1.436	AG3-PT
SJVC1	0.018	0.002	0.042	0.294	1.436	AG3-PT
NCCH3	0.019	0.012	0.025	0.079	1.868	AG3-PT
SRNL2	0.020	0.017	0.023	0.072	2.001	AG3-PT
TJ4.3	0.021	0.013	0.027	0.073	2.075	AG3-PT
SJVC2	0.021	0.009	0.033	0.14	2.096	AG3-PT
TJ2.1	0.021	0.010	0.032	0.112	2.138	AG3-PT
SO3	0.021	0.019	0.024	0.06	2.145	AG3-PT
CA8	0.022	0.015	0.028	0.052	2.176	AG3-PT
SO4	0.022	0.019	0.024	0.052	2.184	AG3-PT
NCCH4	0.022	0.013	0.03	0.088	2.193	AG3-PT
TJ3.1	0.022	0.019	0.025	0.075	2.248	AG3-PT
PC4	0.023	0.011	0.03	0.063	2.255	AG3-PT
A79	0.023	0.020	0.026	0.076	2.296	AG3-PT
SO9	0.024	0.020	0.027	0.088	2.350	AG3-PT
NCCH6	0.024	0.017	0.03	0.078	2.370	AG3-PT
TJ1.1	0.024	0.021	0.026	0.063	2.385	AG3-PT
TJ4.2	0.024	0.021	0.027	0.073	2.410	AG3-PT
SO8	0.025	0.015	0.034	0.122	2.451	AG3-PT
NCCH5	0.026	0.023	0.028	0.067	2.552	AG3-PT
TJ1.3	0.026	0.020	0.031	0.066	2.570	AG3-PT
SO2	0.026	0.023	0.029	0.086	2.605	AG3-PT
PC3	0.027	0.013	0.039	0.118	2.656	AG3-PT
TJ4.1	0.027	0.018	0.036	0.106	2.740	AG3-PT
PNL1	0.028	0.020	0.036	0.135	2.766	AG3-PT
1S	0.029	0.025	0.032	0.123	2.850	AG3-PT
NCCH1	0.029	0.013	0.045	0.180	2.857	AG3-PT
A77	0.029	0.025	0.033	0.132	2.915	AG3-PT
TJ2.2	0.032	0.026	0.038	0.105	3.222	AG3-PT
SJVC3	0.033	0.026	0.041	0.075	3.345	AG3-PT
TJ3.3	0.038	0.025	0.052	0.235	3.784	AG3-PT
SO6	0.027	0.019	0.035	0.103	2.724	AG3-PT
CA7	0.017	0.010	0.024	0.095	1.739	AG1-1B
STNL1	0.027	0.023	0.031	0.163	2.691	AG1-1B
SRNL4	0.030	0.016	0.045	0.187	3.9047	AG1-1B
PNL2	0.039	0.031	0.048	0.126	3.945	AG1-1B
A26	0.020	0.010	0.028	0.107	1.955	AG11

IC = Inhibition concentration, LFL = Lower Fiducial Limits, UFL = Upper Fiducial Limits, RF = Resistance Factor, AG = Anastomosis Group

With the thiabendazole fungicide, *R. solani* strains showed IC_{50} values lesser than 2.91 mg a.i./L, this suggests that all tested strains are susceptible. According to Leach & Murdoch (1985), a

strain is susceptible to thiabendazole if the IC_{50} value is less than 3 mg/L, as in this study (Table 3). Results of this study coincide with those reported by Holguin (1999), who did not find any *R. Solani* strains that were resistant to this fungicide.

Table 3 Inhibitory Concentrations at 50 and 90% (IC_{50} and IC_{90}) and resistance factors to thiabendazol fungicide in *Rhizoctonia solani* strains from different Mexican potato regions.

Strains	IC_{50} (mg/L)	LFL	UFL	IC_{90} (mg/L)	RF	AG
PC4	0.82	0.62	1	2.54	0.27	AG3-PT
TJ2.1	0.88	0.72	1.03	1.81	0.29	AG3-PT
TJ3.1	0.91	0.72	1.09	1.96	0.30	AG3-PT
SJVC2	0.93	0.69	1.15	2.5	0.31	AG3-PT
A31	0.93	0.77	1.08	1.86	0.31	AG3-PT
SO9	0.92	0.79	1.04	1.98	0.31	AG3-PT
SO3	0.94	0.68	1.18	1.83	0.31	AG3-PT
A77	0.96	0.77	1.15	2.41	0.32	AG3-PT
SO4	0.96	0.90	1.01	2.35	0.32	AG3-PT
CA8	0.99	0.79	1.18	1.84	0.33	AG3-PT
NCCH5	0.98	0.67	1.26	2.01	0.33	AG3-PT
NCCH6	0.99	0.87	1.10	2.35	0.33	AG3-PT
TJ4.3	0.99	0.84	1.13	2.41	0.33	AG3-PT
SO8	0.99	0.82	1.16	2.36	0.33	AG3-PT
SO2	1	0.82	1.17	2.22	0.33	AG3-PT
CA1	1.01	0.82	1.18	2.5	0.34	AG3-PT
NCCH4	1.06	1.01	1.12	2.43	0.35	AG3-PT
SJVC1	1.05	1	1.10	2.29	0.35	AG3-PT
1S	1.06	1.01	1.12	2.62	0.35	AG3-PT
TJ4.1	1.06	0.87	1.24	1.93	0.35	AG3-PT
PNL1	1.06	0.73	1.38	2.39	0.35	AG3-PT
TJ1.1	1.04	0.77	1.29	2.4	0.35	AG3-PT
TJ1.3	1.11	0.9	1.32	2.63	0.37	AG3-PT
SRNL2	1.12	0.91	1.33	2.91	0.37	AG3-PT
NCCH1	1.14	0.95	1.33	2.87	0.38	AG3-PT
NCCH3	1.15	1.04	1.27	2.61	0.38	AG3-PT
TJ3.3	1.14	0.85	1.42	2.43	0.38	AG3-PT
PC3	1.18	1.01	1.36	2.44	0.39	AG3-PT
SO6	1.16	1.05	1.27	2.44	0.39	AG3-PT
A79	1.19	1.14	1.24	2.45	0.40	AG3-PT
TJ2.2	1.19	0.99	1.38	3	0.40	AG3-PT
TJ4.2	1.19	0.9	1.5	2.54	0.40	AG3-PT
SJVC3	1.34	1.03	1.65	2.8	0.45	AG1-1B
CA7	0.87	0.71	1.01	1.9	0.29	AG1-1B
SRNL1	2.06	1.73	2.49	5.79	0.69	AG1-1B
PNL2	2.43	2.05	3.05	31.5	0.81	AG1-1B
SJVC4	2.91	2.4	3.73	6.07	0.97	AG1-1B
A26	0.98	0.92	1.03	2.28	0.33	AG11

IC = Inhibition concentration, LFL = Lower Fiducial Limits, UFL = Upper Fiducial Limits, RF = Resistance Factor, AG= Anastomosis Group

Genetic Variability of *Rhizoctonia solani*

The analysis of molecular variance (AMOVA) detected significant differences among origin groups (localities and/or States) (P-value 0.03) and within these (isolates) (P-value 0.025).

Polymorphism analysis included 28 bands obtained by REP-PCR of 48 isolates from *R. Solani* from five potato producing Mexican States, including two reference groups (one from own collection, and ATTC® strains) a variation range between 0.43 (Chihuahua) to 0.86 polymorphic loci from reference strains was observed (ATCC® 76106™ and ATCC® 76129™), with a total value of 0.89 (Table 4).

Table 4 Genetic variability descriptors of *Rhizoctonia solani* isolates.

Statistic	Isolates							Total
	1	2	3	4	5	6	7	
Polymorphic loci	0.68	0.79	0.43	0.71	0.86	0.75	0.75	0.89
Genetic Diversity	0.23	0.28	0.17	0.23	0.34	0.29	0.25	0.29
Unbiased Heterozygosis (Nei)	0.25	0.31	0.19	0.24	0.37	0.31	0.26	0.30
Allele average	1.68	1.79	1.43	1.71	1.86	1.75	1.75	2.00
Effective allele	1.39	1.47	1.30	1.38	1.59	1.50	1.40	1.47

1 = Coahuila, 2 = Canada, 3 = Chihuahua, 4 = Jalisco, 5 = Reference, 6 = Nuevo León, 7 = Sonora

Discussion

Morphological characteristics

All isolates showed typical features of *R. solani* complex (Fig. 2) including brown pigmentation of hyphae, branching near distal septum, constriction of hyphae and formation of a septum at a short distance from the place of branching, and the presence of dolipore septa, and multinuclear cells in young vegetative hyphae (Parmeter & Whitney 1970).

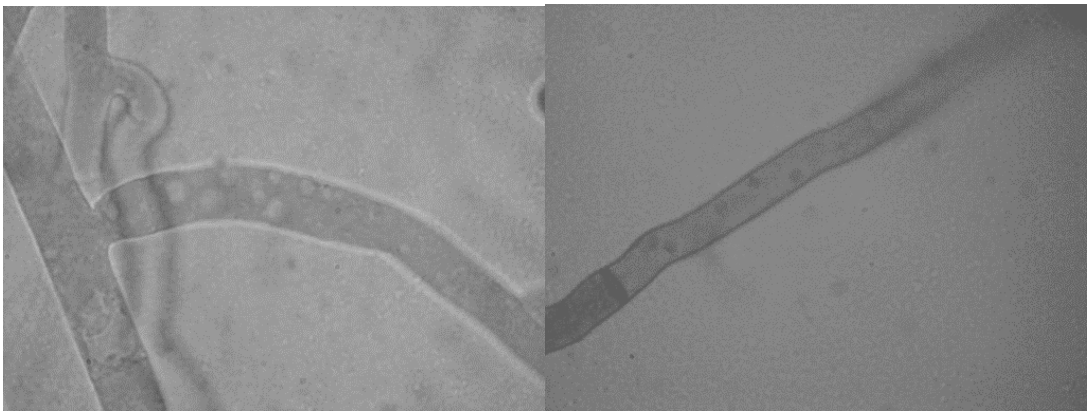

Fig. 2 – Morphological typical characteristic and multinuclear cells of *Rhizoctonia solani.*

Anastomosis group characterization

These results are consistent with most reports, indicating that GA-3 is the group most commonly associated with potato crop (Bandy et al. 1988, Carling & Leiner 1990). However, there are reports on other anastomosis groups such as AG-1, AG-2-1, AG-2-2, AG-4, AG-5 and AG-9 that are attacking potato crop (Carling & Leiner 1990). Other authors have reported for Mexico, that AG-3, AG-2, AG-4, AG-5 and AG-7 groups attacked potato crop; and these groups were identified by the confrontation technique (Holguin 1999, Virgen et al. 2000, Hernández et al. 2001, 2005). This is the first report about AG11 and AG1-1B anastomosis groups associated with potato in Mexico. The highest incidence of AG-3 in potato can be related to its origin associated with sclerotic tissue on the tuber, where *R. solani* isolates were obtained for this study, these results seem to agree with those mentioned by Platt et al. (1993), who reported AG-3 isolates from tubers and other anastomosis groups different to AG-3 when isolates are obtained from soil or infected stems. Similar results are reported by Montero et al. (2013). These authors isolated different AG

groups from pepper where stem and roots were used. They mainly isolated fungal strains belonging to AG-4, AG-7, and AG-13 and in minor proportion isolates belonging to AG-12, AG-11, AG2-2IV, AG-2-2IIIB and AG-2-1 groups.

Anastomosis groups of *Rhizoctonia solani* and their resistance to fungicides

The RF values were higher than the unit, so it is considered that the *R. solani* isolates were resistant to pencycuron (Koller & Scheinpflug 1987, Leroux 1987). Resistance to pencycuron has been reported by other authors. *R. solani* strains belonging to AG-3 group and isolated from potatoes from Chihuahua showed IC_{50} from 0.002 to 1.04 (Hernández et al. 2005) and *R. solani* strains isolated from different regions of Mexico, also showed resistance to pencycuron (Holguin 1999, Chávez et al. 2011).

Several reports indicated that there are differences among anastomosis groups on tolerance to both fungicides evaluated in this study. It is observed that the anastomosis group more tolerant to both fungicides is AG-11; this behavior has been previously reported by several authors, indicating that each anastomosis group tolerates different concentrations of fungicides (Lehtonen et al. 2008a). For the pencycuron fungicide, Hernández et al. (2005) and Chávez et al. (2011) reported a RF for the AG-4 group of 225.9 and 104.4 for AG-3. These values are higher than those observed in this study, but the tendency is the same.

Genetic Variability of *Rhizoctonia solani*

The population used as a reference, showed the highest genetic diversity value (0.34) (Table 4); this is because its individuals are genetically distinct (AG-6, AG-8, AG-3, and AG-11), while the Chihuahua population showed the lowest genetic variability (0.17), which may be due to Chihuahuan regions remained isolated from introduction of potato seed tuber from Canada in the early 90's, so there are fewer genotypes of *Rhizoctonia*. Moreover, Chihuahua is the oldest region where potato is planted in Mexico, so individuals that are less fit may disappear, thus reducing genetic variability. Also, the lowest values of unbiased heterozygotes were showed by the Chihuahuan population with 0.19, and the highest values by the reference (GA-6 and GA-8) population with 0.37. The average number of alleles ranged from 1.43 to 1.86 among populations. The highest genetic variability was observed in the control group (0.34), which is given because in this group have different anastomosis groups, in contrast, the population from Chihuahua had the lowest (0.17) variability, where all *R. solani* isolates were identified as belonging to the same anastomosis group. Genetic diversity was very low in most populations; this is attributed to that this fungus spreads asexually (mycelium and sclerotic tissue which spreads through seed tuber). There are few reports of parasexual cycle in *R. solani*, which has not been completely deciphered for this species, even though, it is a phenomenon with some occurrence (Parameter, 1970). According to the results generated in this study, genetic variability of *R. solani* in the studied populations could be generated only by mutation and gene flow.

The average genetic diversity observed in this study (0.29) is similar to that of 0.2945 reported by Ceresini et al. (2002), also a low value of this measure, reflects low recombination, so that, the greatest effects of asexual reproduction is production of few recombinant genotypes, and low genetic diversity. Although, the occurrence of duplicate genotypes was not observed, the low genetic diversity may indicate a clonal reproduction of the analyzed isolations, and low parasexual cycle. In the analysis of molecular variance (Table 5), significant differences between (p-value 0.03) and within populations (*p*-value 0.02) were found, where genetic variability among populations was two-fold higher than variability within populations. We consider a population as derived from locality, where each population is adapted to climatic conditions of the zone and each zone has specific characteristics, so this genetic variability is due in response to adaptive evolution. In contrast, the high similarity observed within populations can be due that were isolating few anastomosis groups among the population, and they express heightener similarity of allele. In addition; therefore, it is likely that less diversity exists among the strains analyzed in the same population (Saavedra & Spoor 2002).

Table 5 Analysis of molecular variance (AMOVA) among anastomosis groups.

Variation	SS	FD	MS	P-VALUE	Number of interactions
Population	13.43	1	13.43	<0.03	400
Within	316.15	41	7.71	<0.0225	400
Total	329.58	42	7.85		

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