Diversity of *Pyrenophora tritici-repentis* isolates from the Argentinian wheat growing area: morphocultural and pathogenic analysis

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Wheat is currently considered as one of the most important crops world-wide. Among the fungal diseases of this crop, tan spot produced by *Pyrenophora tritici-repentis* is one of the most important. Tan spot increased its incidence, prevalence and severity in Argentina and South America. The aim of this work was to generate information about the morphocultural and pathogenic variability on wheat cultivars from Argentina. The results showed that isolates of *P. tritici-repentis*, originated from diverse localities of Argentina differed in their morphocultural characteristics and in their level of severity. The cluster analysis of morphocultural characteristics among 155 isolates of the pathogen defined 44 morphotypes according with the Jaccard's coefficient (CCC=0.79). Pathogenicity tests determined on a set of eight wheat cultivars under greenhouse conditions showed the presence of physiological specialization in 33 isolates of *P. tritici-repentis*. The efforts to link morphocultural and pathogenicity features to the geographical origin of the isolates were mostly unsuccessful. The isolates tested appeared in different groups and most of the isolates which shared the highest similarity coefficient were collected from different localities and different wheat cultivars.

Keywords: Morphocultural, pathogenicity, *Pyrenophora tritici-repentis*, tan spot, variability, wheat.

INTRODUCTION

Wheat is one of the most important crops, so long 100 years in Argentina. Among the years 2009/2010 was cultivated 5 million ha and yield was among 7.5 million tons. The future of this production and the participation of South America in the international markets depended of the evolution of proposal of Argentina and Brazil. In Argentina, the start in the field and the exportation of the crop depended in the introduction of new technological develops. These develop are the new cultivar composition, management of crops and management of future areas for yield expanding (Eikboir and Morris, 2001). The pathogens fungi are become by the combination of these factors (Klein, 2001). Globally, an estimated 25 million ha of wheat are affected by *Pyrenophora tritici-repentis* (Duveiller et al., 2005). Tan spot, caused by *P. tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem., is an economically important disease of wheat (Wiese, 1987). Tan spot reduces total yield, kernel weight (Shabeer and Bockus, 1988; Schilder and Bergstrom, 1990), number of grains per head (Schilder and Bergstrom, 1990), total biomass (Kremer and Hoffmann, 1992), and/or grain quality because of red-smudge symptoms (Fernandez et al., 1994). The shift in farming practices toward the stubble retention has resulted in a considerable increase in disease incidence in major wheat growing areas

(Hosford, 1982). Yield losses have ranged from 3% to 53%, depending on the cultivar susceptibility, environmental conditions, and virulence of the pathogen population (Hosford, 1971; Rees and Platz, 1982). Due to changes in cultural practices (e.g., minimum or zero tillage), the cultivation of susceptible varieties, and changes in the virulence of the pathogen, tan spot has become a serious concern for wheat producers in the Southern Cone region of South America including Argentina, Brazil, Chile, Paraguay and Uruguay (Kohli, 1995).

In the early 1980s, *P. tritici-repentis* was found at first time affecting wheat crops in the north-central region of Buenos Aires, Argentina (Annone, 1985). Subsequently, tan spot has gained predominance among foliar wheat diseases in most of the Agentinean wheat growing areas (Annone, 1995; Carmona et al., 1999). Increase in the severity of the disease has been linked with the change in the pathogen virulence, wide adoption of non-till and conservation tillage practices and/or introduction of susceptible cultivars (Ali and Francl, 2002; Klein, 2001). It is commonly accepted that changing agronomic practices toward soil conservation have resulted in increased inoculum levels and higher disease incidence (Rees and Platz, 1982).

P. tritici-repentis has conidiophores arising singly or in groups of 2-3, emerging through stomata or between epidermal cells, erect, simple, straight or flexuous, sometimes geniculate, cylindrical or slightly tapered, often swollen at the base, mid-pale to brown, smooth, usually up to 250 µ long but occasionally one or a few rather inconspicuous conidial scars. Conidia solitary, straight or slightly curved, cylindrical, rounded at the apex, the basal segment distinctly and characteristically conical or shape of a snake's head, typically subhyaline or rather pale straw-coloured, smooth, thin-walled, with 1-9 (usually 5-7) pseudosepta, old conidia often constricted at the pseudosepta, 80-250 (117) µ lon, 14-20 (17.7) µ thick in the broadest part, 2-4 (3) µ wide at the base (Ellis and Waller, 1976). Colony consist of low, dense, white to medium grey mycelium covering about two–thirds of the slant surface, and slightly in advance of the growth on the surface (Shoemarker, 1962).

Diaz de Ackermann, (1987), Hosford, (1971) and Hunger and Brown, (1987), working with *P. tritici-repentis* isolated from different locations, observed some variability in mycelium color and colony morphology. Differences in the colony diameter of the fungus were reported in others studies (Gilchrist et al., 1984).

Isolates of *P. tritici-repentis* have also been reported to differing in virulence (Cox and Hosford, 1987; Diaz de Ackermann, 1987; Gilchrist et al., 1984; Krupinsky 1987, 1992 a,b; Lamari and Bernier, 1989a; Luz and Hosford, 1980; Misra and Singh, 1972; Nagle et al., 1982;). The tan spot syndrome consists of two phenotypically distinct and independent symptoms: tan necrosis and extensive chlorosis (Lamari and Bernier, 1989a,b). Isolates of *P. tritici repentis* are now grouped into races on the basis of their virulence on individual host differential genotypes (Ali and Francl, 2001, 2002, 2003; Lamari et al., 2003; Manning et al., 2002). Lamari et al. (1995) proposed a race-based system to describe isolates of *P. tritici-repentis* and currently eight pathotypes have been identified (Ali and Francl, 2001, 2002, 2003; Lamari et al., 2003; 2005; Manning et al., 2002). According to Ali and Francl, (2002), *P. tritici-repentis* has a diverse population in South America.

The management of tan spot disease involves the ability to identify the fungus and the use of techniques for reduced to minimum the losses of crop (Kohli 1995). In the argentinian growing area, peudothecia of the pathogen are found on wheat residue left on the soil surface at crop sowing and/or at early growth stages. *P.tritici-repentis* is known to have a very complex interaction with its host. Previous results (Moreno et al., 2008 a,b; Moreno et al., unpublished data) about argentinean population of *P. tritici-repentis* is complex in reaction types and this characteristic should be considered.

The present work had as main, know the variations within of *Pyrenophora tritici repentis*'s population, the morphological aspects and the variability of the virulence on wheat cultivars in Argentina.

MATERIALS AND METHODS

Fungal isolates

The leaf samples were collected from 13 wheat cultivars and *Festuca* sp. from 23 localities of different regions of Argentina, in years 2000, 2001, 2002 and 2003 (Figure 1). Leaves of each collected samples were cut into 1.0-1.5 cm pieces so that a lesion was cut through the center. Five pieces of each leaf were selected at random, washed under tap water, surface-sterilized by dipping successively into 70% ethanol for 2 min, 5% sodium hypoclorite (commercial 55 g Cl/L) for 2 min and finally rinsed twice in fresh sterilized distilled water. Five leaf pieces were placed in each Petri dish containing Potato Dextrose Agar 2% (PDA) acidified to pH 5 with 250 mg chloramphenicol/L to suppress bacterial growth. Plates were incubated for 4 or 5 days at 21º± 2ºC with light alternating (3500 lux dark cycles of 12 h plus the addition of near UV light 365nm). The isolates were identified according to species concepts and descriptions, and taxonomic standards for species separation (Ellis and Waller, 1976). One hundred and fifty-four isolates of *P. tritici-repentis* were obtained from leaves of wheat and one isolate (FH041) was obtained from leaves of *Festuca* sp. (Table 1). The isolates were stored under sterile mineral oil at 4ºC in the culture collection of the Centro de

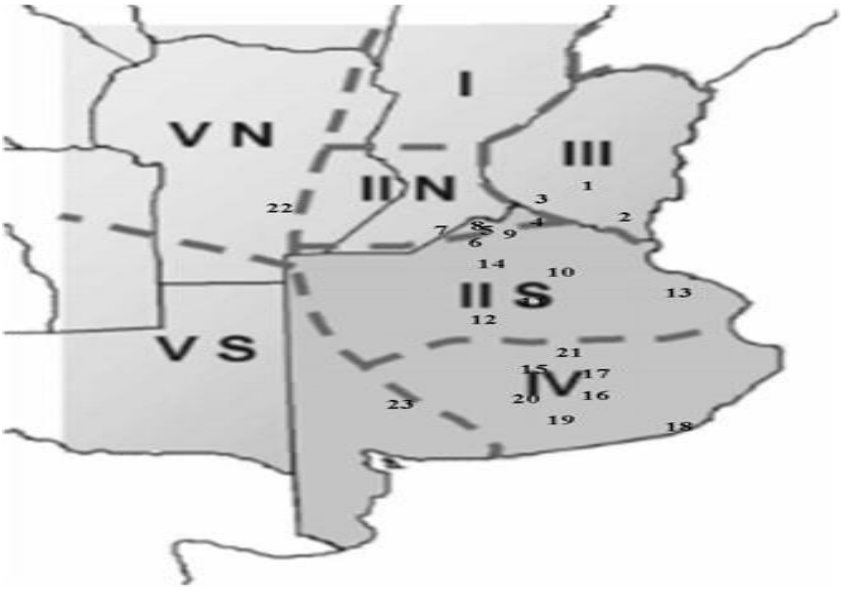

Figure 1. Map of areas of wheat in Argentina, showing the localities where samples of the *P. tritici-repentis* isolates were collected.
References figure 1 I, II North, II South, III, IV, V North and V South : Areas of wheat in Argentina Localities: 1: Rincón Nogoyá, 2: Gualeguaychú, 3: Victoria, 4: Salto, 5: Pergamino, 6: Arrecifes, 7: Comodoro Py, 8: Chivilcoy, 9: Arroyo Dulce, 10: Alberti, 11: 9 de Julio, 12: 25 de Mayo, 13: Los Hornos, 14: Bragado, 15: Olavaria, 16: Tandil, 17: Azul: 18: Orense, 19: Benito Juárez, 20: Chillar, 21: Tapalqué, 22: Marcos Juárez, 23: Coronel Suárez

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Colony and conidial morphology

One hundred and fifty-five single conidial isolates of *P. tritici-repentis* were transferred to 2% PDA and V8-agar (200 ml of V8 juice®, 3 g $CaCO_3$, 20 g agar and 800 ml distilled water) plates under different conditions of light cycles in order to observed colony colour and characteristics in culture. The treatments were: TI: 2% PDA 12 h light-dark; TII: 2% PDA continuous dark; TIII: V8-agar 12 h light-dark and TIV: V8-agar continuous dark, all incubated for 7 days at 21º$\pm$ 2ºC. At the 7th day of incubation the dishes were then incubated in continuous light for 24 h and and 24 h dark to induce the formation of conidiophores and conidia (Odvody and Boosalis, 1982).

Colony fungal variability (colour, border, texture and diameter) were recorded. To determine the conidia morphology, one hundred conidia were measured for length and number of septa/conidia for each isolate. To determine the sporulation, the conidial harvest was made by flooding the plate with 5 ml of sterile distilled water and dislodging the conidia with a bent glass rod. The resulting suspension was filtered through cheesecloth. The number of conidia mm^{-2} was determined with the Neubawer hemocytometer.

Pathogenicity test

The inoculum preparation, inoculum procedure and the pathogenicity test were made according to Moreno et al (2008b). A differential set was selected within quite of the most commercial wheat cultivars used in Argentina. (ACA 303, ACA 801, Baguette 10, Buck Premiun 13, Klein Chaja, K. Escorpion, K. Flecha and K. Jabali).

Statistical analysis

Cultures, colony and conidial morphology

The macroscopic characters (colour, border and texture of colony) of each isolate were analyzed as frequency. Data of colony diameter were analyzed to an ANOVA using the computer programe MSTAT-c (Rusell). Means were compared with the Tukey's test. Characters, as number of septa/conidia, length of conidia, number of conidia mm^{-2} and the average of diameter of colony for the isolates cultivated in TIII, were considered in the

Table 1. Origin of *Pyrenophora tritici-repentis* isolates examined in this study.

Isolates	Nomenclature	Localities	Cultivar	Wheat Area	Year
A1	CP021	Comodoro Py	Baguette 10	II Norte	2002
A2	OL022	Olavarria	Klein Don Enrique	IV	2002
A3	TA022	Tandil	Baguette 10	IV	2002
A4	AZ021	Azul	Klein Don Enrique	IV	2002
A5	AZ023	Azul	Klein Don Enrique	IV	2002
A6	AZ024	Azul	Klein Don Enrique	IV	2002
A7	O001	Orense	Buck Sureño	IV	2000
A8	O0020	Orense	Buck Sureño	IV	2000
A9	O0012	Orense	Buck Sureño	IV	2000
A10	O0014	Orense	Buck Sureño	IV	2000
A11	O0019	Orense	Buck Sureño	IV	2000
A12	O0017	Orense	Buck Sureño	IV	2000
A13	O0015	Orense	Buck Sureño	IV	2000
A14	P021	Pergamino	Klein Don Enrique	II Norte	2002
A15	P022	Pergamino	Klein Don Enrique	II Norte	2002
A16	P023	Pergamino	Klein Don Enrique	II Norte	2002
A17	P026	Pergamino	Klein Don Enrique	II Norte	2002
A18	P027	Pergamino	Klein Don Enrique	II Norte	2002
A19	P028	Pergamino	Klein Don Enrique	II Norte	2002
A20	P031	Pergamino	Klein Don Enrique	II Norte	2003
A21	P032	Pergamino	Klein Don Enrique	II Norte	2003
A22	P0313	Pergamino	Klein Don Enrique	II Norte	2003
A23	CK032	Alberti	Klein Escudo	II Sur	2003
A24	CK033	Alberti	Klein Escudo	II Sur	2003
A25	CK034	Alberti	Klein Escudo	II Sur	2003
A26	S031	Salto	Klein Escorpión	II Norte	2003
A27	S032	Salto	Klein Escorpión	II Norte	2003
A28	CHI0313	Chivilcoy	Klein Escorpión	II Norte	2003
A29	MJ032	Marcos Juárez	Klein Escorpión	V Norte	2003
A30	AD031	Arroyo Dulce	Baguette 10	II Norte	2003
A31	9J031	9 de Julio	Klein Escorpión	II Sur	2003
A32	9J032	9 de Julio	Klein Escorpión	II Sur	2003
A33	ARR031	Arrecifes	Buck Mataco	II Norte	2003
A34	ARR034	Arrecifes	Buck Mataco	II Norte	2003
A35	ARR035	Arrecifes	Buck Mataco	II Norte	2003
A36	25M031	25 de Mayo	Buck Mataco	II Sur	2003
A37	25M032	25 de Mayo	Buck Mataco	II Sur	2003
A38	25M033	25 de Mayo	Buck Mataco	II Sur	2003
A39	25M034	25 de Mayo	Buck Mataco	II Sur	2003
A40	25M035	25 de Mayo	Buck Mataco	II Sur	2003
A41	25M036	25 de Mayo	Buck Mataco	II Sur	2003
A42	25M037	25 de Mayo	Buck Mataco	II Sur	2003
A43	H0124	Los Hornos	Buck Brasil	II Sur	2001
A44	H0100	Los Hornos	Buck Brasil	II Sur	2001
A45	H0110	Los Hornos	Buck Brasil	II Sur	2001
A46	H0113	Los Hornos	Buck Brasil	II Sur	2001
A47	H0114	Los Hornos	Buck Brasil	II Sur	2001
A48	H0115	Los Hornos	Buck Brasil	II Sur	2001
A49	H003	Los Hornos	Buck Brasil	II Sur	2000
A50	H006	Los Hornos	Buck Brasil	II Sur	2000
A51	H008	Los Hornos	Buck Brasil	II Sur	2000
A52	H0011	Los Hornos	Buck Brasil	II Sur	2000
A53	H0014	Los Hornos	Buck Brasil	II Sur	2000

Table 1 continue

Isolates	Nomenclature	Localities	Cultivar	Wheat Area	Year
A54	H0017	Los Hornos	Buck Brasil	II Sur	2000
A55	H0019	Los Hornos	Buck Brasil	II Sur	2000
A56	BJ0011	Benito Juárez	Klein Don Enrique	IV	2000
A57	CS002	Coronel Suárez	Klein Don Enrique	V Sur	2000
A58	A021	Alberti	Klein Escudo	II Sur	2002
A59	CH002	Chillar	Klein Don Enrique	IV	2000
A60	CH004	Chillar	Klein Don Enrique	IV	2000
A61	CH005	Chillar	Klein Don Enrique	IV	2000
A62	CH006	Chillar	Klein Don Enrique	IV	2000
A63	CH007	Chillar	Klein Don Enrique	IV	2000
A64	CH008	Chillar	Klein Don Enrique	IV	2000
A65	CH009	Chillar	Klein Don Enrique	IV	2000
A66	T006	Tapalqué	Klein Don Enrique	IV	2000
A67	T0012	Tapalqué	Klein Don Enrique	IV	2000
A68	T0017	Tapalqué	Klein Don Enrique	IV	2000
A69	N021	Rincón Nogoyá	Baguette 10	III	2002
A70	N022	Rincón Nogoyá	Baguette 10	III	2002
A71	V021	Victoria	Baguette 10	III	2002
A72	V022	Victoria	Baguette 10	III	2002
A73	V0212	Victoria	Baguette 10	III	2002
A74	V0214	Victoria	Baguette 10	III	2002
A75	B022	Bragado	Klein Escorpión	II Sur	2002
A76	B023	Bragado	Klein Escorpión	II Sur	2002
A77	B025	Bragado	Klein Escorpión	II Sur	2002
A78	B026	Bragado	Klein Escorpión	II Sur	2002
A79	B029	Bragado	Klein Escorpión	II Sur	2002
A80	G031	Gualeguaychú	Baguette 10	III	2003
A81	G032	Gualeguaychú	Baguette 10	III	2003
A82	G035	Gualeguaychú	Baguette 10	III	2003
A83	G036	Gualeguaychú	Buck Biguá	III	2003
A84	G037	Gualeguaychú	Buck Biguá	III	2003
A85	G038	Gualeguaychú	Buck Biguá	III	2003
A86	G039	Gualeguaychú	Buck Biguá	III	2003
A87	G0310	Gualeguaychú	Buck Mataco	III	2003
A88	G0311	Gualeguaychú	Buck Mataco	III	2003
A89	G0312	Gualeguaychú	Buck Mataco	III	2003
A90	G0313	Gualeguaychú	Buck Mataco	III	2003
A91	G0315	Gualeguaychú	Buck Guapo	III	2003
A92	G0316	Gualeguaychú	Buck Guapo	III	2003
A93	G0317	Gualeguaychú	Buck Guapo	III	2003
A94	G0318	Gualeguaychú	Buck Guapo	III	2003
A95	G0319	Gualeguaychú	Buck Guapo	III	2003
A96	G0321	Gualeguaychú	Klein Chajá	III	2003
A97	G0322	Gualeguaychú	Klein Chajá	III	2003
A98	G0323	Gualeguaychú	Klein Chajá	III	2003
A99	G0324	Gualeguaychú	Klein Chajá	III	2003
A100	G0325	Gualeguaychú	Klein Zorzal	III	2003
A101	G0326	Gualeguaychú	Klein Zorzal	III	2003
A102	G0327	Gualeguaychú	Klein Zorzal	III	2003
A103	G0328	Gualeguaychú	Klein Zorzal	III	2003
A104	G0329	Gualeguaychú	Klein Zorzal	III	2003
A105	G0330	Gualeguaychú	Klein Zorzal	III	2003
A106	G0331	Gualeguaychú	INTA Tjereta	III	2003

Table 1 continue

Isolates	Nomenclature	Localities	Cultivar	Wheat Area	Year
A107	G0332	Gualeguaychú	INTA Tijereta	III	2003
A108	G0334	Gualeguaychú	Klein Churrinche	III	2003
A109	G0336	Gualeguaychú	Klein Zorzal	III	2003
A110	TA024	Tandil	Baguette 10	IV	2002
A111	O008	Orense	Buck Sureño	IV	2000
A112	V025	Victoria	Baguette 10	III	2002
A113	V024	Victoria	Baguette 10	III	2002
A114	BJ00	Benito Juárez	Klein Escorpión	IV	2000
A115	TA021	Tandil	Baguette 10	IV	2002
A116	CS001	Coronel Suárez	Klein Don Enrique	V Sur	2000
A117	A029	Alberti	Klein Escudo	II Sur	2002
A118	B021	Bragado	Klein Escorpión	II Sur	2002
A119	V0213	Victoria	Baguette 10	III	2002
A120	A0210	Alberti	Klein Escudo	II Sur	2002
A121	AZ022	Azul	Klein Don Enrique	IV	2002
A122	H021	Los Hornos	Buck Brasil	II Sur	2002
A123	T009	Tapalqué	Klein Don Enrique	IV	2000
A124	T008	Tapalqué	Klein Don Enrique	IV	2000
A125	T001	Tapalqué	Klein Don Enrique	IV	2000
A126	H004	Los Hornos	Buck Brasil	II Sur	2000
A127	H0018	Los Hornos	Buck Brasil	II Sur	2000
A128	O0011	Orense	Buck Sureño	IV	2000
A129	BJ0012	Benito Juárez	Klein Don Enrique	IV	2000
A130	H007	Los Hornos	Buck Brasil	II Sur	2000
A131	H0012	Los Hornos	Buck Brasil	II Sur	2000
A132	H001	Los Hornos	Buck Brasil	II Sur	2000
A133	G034	Gualeguaychú	Baguette 10	III	2003
A134	G0300	Gualeguaychú	Baguette 10	III	2003
A135	G0333	Gualeguaychú	Klein Churrinche	III	2003
A136	G0320	Gualeguaychú	Klein Chajá	III	2003
A137	MJ031	Marcos Juárez	Klein Escorpión	V Norte	2003
A138	B033	Bragado	Klein Escorpión	II Sur	2003
A139	B0210	Bragado	Klein Escorpión	II Sur	2002
A140	B027	Bragado	Klein Escorpión	II Sur	2002
A141	B024	Bragado	Klein Escorpión	II Sur	2002
A142	H0119	Los Hornos	Buck Brasil	II Sur	2001
A143	H0120	Los Hornos	Buck Brasil	II Sur	2001
A144	B028	Bragado	Klein Escorpión	II Sur	2002
A145	H016	Los Hornos	Buck Brasil	II Sur	2001
A146	H013	Los Hornos	Buck Brasil	II Sur	2001
A147	H012	Los Hornos	Buck Brasil	II Sur	2001
A148	H015	Los Hornos	Buck Brasil	II Sur	2001
A149	H0111	Los Hornos	Buck Brasil	II Sur	2001
A150	O0018	Orense	Buck Sureño	IV	2000
A151	CH0010	Chillar	Klein Don Enrique	IV	2000
A152	C041	Alberti	Klein Don Enrique	II Sur	2004
A153	C042	Alberti	Klein Don Enrique	II Sur	2004
A154	C043	Alberti	Klein Don Enrique	II Sur	2004
A155	FH041	Los Hornos	*Festuca* sp	II Sur	2004

similarity analysis. The quantitative characters used were subdivided in intervals for the purpose to obtain characters binary, for example number of septa/conidia: 4-6 is present (1) or number of septa/conidia 4-6 is absent (0); septa/conidia: 7-9 is present (1) or number of septa/conidia 7-9 is absent (0); the same for the others characters.

The data were assembled in a matrix for similarity analysis, and the Jaccard's similarity coefficient (Sneath and Sokal, 1973) was computed for all possible pairs of isolates. The similarity index of Jaccard between i and j is given by Si=a/(a+b+c+), where a is the number of characters present in both i and j, b is the number of characters present in i but not in j, and c the number of the characters present in j but not in i. The matrix of similarity coefficient was then subjected to cluster analysis by using the unweighted pair-group method with arithmetic averages (UPGMA). The Cophenetic Correlation coefficient (CCC) was chosen to indicate the level of distortion between the similarity matrix and cluster analysis. NTSyS-pc version 2.0 was used to perform these analyses.

Pathogenicity test

Data of disease severity (percent leaf area infected) were arcsine square-root transformed and were submitted to an ANOVA analysis using the computer program MSTAT-c (Russell & Einsensmith). Means presented as real values were separated by Tukey´s test (P=0.05). The model used for the ANOVA was: Y_{ijk}: $\mu + \tau_i + \varepsilon_{ij} + \eta_{ijk}$; where τ_i is the effect of the region of origin, ε_{ij} is the contingent error associated with each observation, and η_{ijk} is the error associated with the sub sample independently of ε_{ij}.

RESULTS

Colony and conidial morphology

The colony characteristics varied considerably between the *P. tritici-repentis* isolates and treatments (Figure 2 and Table 2). The colony colour in 2% PDA varied from olivaceus (TI: 71% and TII 70%), olivaceus black (TI: 25% and TII: 24%), olivaceus buff (TI: 2% and TII: 4%) to greenish olivaceus (TI: 1% and TII: 1%). On V8-agar the top surface of the colony colour varied from salmon (TIII: 6% and TIV: 9%), rosy buff (TIII: 35% and TIV: 46%), greenish olivaceus (TIII: 1% and TIV: 3%), buff (TIII: 2% and TIV: 4%) to lavander grey (TIII: 56% and TIV: 38%). The colony colour greenish olivaceous was observed in all treatments. The isolate BJ0012 always presented the colony colour greenish olivaceous independently of the treatment. Some isolates presented a red surface colony.

The most frequently top colony surface observed was smooth. However, in TIII most of the isolates (68%) developed concentric rings. It was attributed to the exposition under light cycles and the culture medium specificity. All isolates developed poor aerial mycelia in TIII and TIV. It was attributed with the fact that these conditions the isolates produced conidia profusely. Some isolates in V8-agar (126 isolates on TIII and 24 isolates on TIV) produced undeveloped pseudothecia.

In TIII, five isolates (AZ023, FH041, N021, N022, TA022) did not produce conidia in culture. Seventy isolates produce 10000 conidia mm^{-2}, forty-six isolates produced 20000 to 40000 conidia mm^{-2} and thirty-three isolates produced more than 40000 conidia mm^{-2}. In TIV only seventy-five isolates of the pathogen did not produce conidia in culture, forty-seven isolates produced 10000 conidia mm^{-2}, thirteen isolates produced between 20000 and 40000 conidia mm^{-2} and only twenty isolates of *P. tritici-repentis* produced that more 40000 conidia mm^{-2}. This suggested that 24 h of light might be not sufficient for conidial production when the isolates are cultivated in continuous dark for 7 days.

The colony diameter was $\geq$ 1 cm/day for the 60% of isolates in TI and 46% of isolates of *P. tritici-repentis* in TII. However, the colony diameter in V8-agar was of 0.50-0.99 cm/day for the 55% of isolates in TIII and the 74% of isolates of *P. tritici-repentis* in TIV. The ANOVA for the colony diameter showed significant differences among the isolates for each (Table 3).

Throughout this report, the term morphotype is used to designate the different isolates groups according to the cluster analysis. Forty four different groups were separated at 100% of similarity (CCC= 0.79) (Figure 3, Table 2). The isolates were grouped in nine subcluster (I, II, III, IV, V, VI, VII, VIII and IX) with 50 % (average) of similarity among groups. The subcluster I included 18 isolates collected from nine localities and eight wheat cultivars. The subcluster II grouped 13 isolates obtained from eight localities and seven wheat cultivars. In the subcluster III we observed seven isolates that were collected from Benito Juárez, Gualeguaychú and Orense and four wheat cultivars. The subcluster IV included 13 isolates obtained from four localities and four wheat cultivars The subcluster V included 35 isolates. It is representing ten localities and ten wheat cultivars. The subcluster VI included 30 isolates of *P. tritici-repentis* collected from ten localities and ten wheat cultivars. The subcluster VII grouped 29 isolates collected from ten localities and ten wheat cultivars. In the subcluster VIII seven isolates were grouped and they were isolated from Arroyo Dulce, Bragado, Chivilcoy, Los Hornos, and 25 de Mayo and five wheat cultivars. The subcluster IX included

Table 2. Morphocultural characteristics used to discriminate among isolates of *Pyrenophora tritici-repentis*.

	Treatment I						Treatment II						Treatment III						Treatment IV					
ISOLATES	**COLOUR**	**SUR**	**EDGE**	**FORM**	**MORP**	**DAM**	**COLOUR**	**SUR**	**EDGE**	**FORM**	**MORP**	**DAM**	**COLOUR**	**SUR**	**EDGE**	**FORM**	**MORP**	**DAM**	**COLOUR**	**SUR**	**EDGE**	**FORM**	**MORP**	**DAM**
CP021	OLV	R	FF	FL	H	T	OLV	R	FF	FL	D	T	LG	RC	F	C	D	M	B	E	FF	FL	D	T
OL022	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	M	B	E	FF	FL	D	T
TA022	OLV	E	F	FL	H	M	OLV	E	F	FL	H	M	LG	RC	FF	C	D	M	LG	E	FF	FL	D	T
AZ021	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	R	FF	FL	P	M
AZ023	OLV	E	FF	FL	H	T	OLV	E	FF	FL	H	M	LG	R	FF	C	D	P	LG	R	FF	FL	P	M
AZ024	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	M	LG	R	FF	C	H	P	RB	R	FF	FL	H	P
O001	OLV	E	F	C	P	M	OLVBF	E	F	C	H	M	LG	RC	FF	C	D	T	B	RC	FF	C	H	P
O0020	OLVB	E	F	C	H	M	OLVB	E	FF	C	H	M	B	RC	FF	C	D	T	B	E	FF	C	H	P
O0012	OLVB	E	F	C	H	P	OLVB	E	FF	C	H	M	B	RC	FF	C	D	T	B	E	FF	C	H	P
O0014	OLVB	E	F	C	H	P	OLVB	E	FF	C	H	M	B	RC	FF	C	D	T	B	E	FF	C	H	P
O0019	OLVB	E	F	C	H	P	OLVB	E	FF	C	H	M	B	RC	FF	C	D	T	B	E	FF	C	H	P
O0017	OLVB	E	F	C	H	M	OLVB	E	FF	C	H	M	B	RC	FF	C	D	T	B	E	FF	C	H	P
O0015	OLVB	E	F	C	H	P	OLVB	E	FF	C	H	M	B	RC	FF	C	D	T	B	E	FF	C	H	P
P021	OLV	R	FF	FL	H	P	OLVB	E	FF	FL	H	P	LG	RC	FF	C	D	M	LG	R	FF	FL	H	P
P022	OLV	E	FF	FL	P	P	OLVB	E	FF	FL	H	P	LG	RC	FF	C	D	M	LG	R	FF	FL	P	M
P023	OLV	R	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	D	T	LG	R	FF	FL	H	P
P026	OLV	R	FF	FL	P	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	D	M	LG	R	FF	FL	H	P
P027	OLV	E	FF	FL	P	P	OLV	E	FF	FL	H	P	B	RC	F	C	D	M	LG	E	FF	FL	H	P
P028	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	R	FF	C	P	M	S	E	FF	FL	D	M
P031	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	R	FF	C	H	M	B	R	FF	FL	P	M
P032	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	R	FF	C	D	M	B	R	FF	FL	P	M
P0313	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	R	FF	C	D	M	B	R	FF	FL	H	P
CK032	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	T	B	E	FF	C	H	P
CK033	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	T	B	E	FF	C	H	P
CK034	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	T	B	E	FF	C	H	P
S031	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	M	B	E	FF	C	H	P
S032	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	M	B	E	FF	C	H	P
CHI0313	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	P	B	E	FF	C	H	P
CK035	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	P	LG	RC	FF	C	D	M	S	R	FF	FL	P	M
MJ032	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	B	R	FF	FL	H	P
AD031	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	FF	C	H	M	B	R	FF	FL	H	P
9J031	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	FF	C	H	M	B	E	FF	FL	H	P
9J032	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	M	B	E	FF	FL	D	M
ARR031	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	M	B	E	FF	C	H	P
ARR034	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	M	B	E	FF	C	H	P
ARR035	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	P	LG	RC	FF	C	H	M	B	R	FF	C	H	P
25M031	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	P	LG	RC	FF	C	H	T	B	R	FF	C	H	P
25M032	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	P	LG	RC	FF	C	H	T	B	R	FF	C	H	P

Table 2 continue

25M033	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	P	LG	RC	FF	C	H	T	B	R	FF	C	H	P
25M034	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	P	LG	RC	FF	C	H	T	B	R	FF	C	H	P
25M035	OLV	E	FF	FL	H	M	OLV	E	FF	FL	H	P	LG	RC	FF	C	H	T	B	R	FF	C	H	P
25M036	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	B	E	FF	C	P	T	B	R	FF	C	H	P
25M037	OLV	E	FF	FL	H	P	OLVB	E	FF	FL	H	M	B	E	FF	C	H	T	B	R	F	FL	H	P
H0124	OLVB	E	FF	FL	H	P	OLVB	E	FF	C	H	M	B	E	FF	C	D	T	B	R	F	FL	H	P
H0100	OLV	E	FF	C	H	P	OLVB	E	FF	C	H	P	B	E	F	C	D	T	B	R	F	C	H	P
H0110	OLVB	E	FF	C	H	M	OLVB	E	FF	C	H	P	B	E	F	C	D	T	B	E	F	C	H	P
H0113	OLVB	E	FF	C	H	M	OLVB	E	FF	FL	H	P	B	E	F	C	D	T	B	E	F	C	H	P
H0114	OLVB	E	FF	FL	H	M	OLVB	E	FF	C	H	P	B	E	F	C	D	T	B	E	F	C	H	P
H0115	OLV	E	FF	C	H	P	OLVB	E	F	C	H	M	B	E	F	C	D	M	B	E	FF	C	H	P
H003	OLVB	E	F	C	H	M	OLVB	E	F	C	H	M	B	E	F	C	D	T	B	E	F	C	H	P
H006	OLVB	E	FF	C	H	M	OLV	E	F	C	H	M	B	E	F	C	D	M	B	E	F	C	H	P
H008	OLV	E	FF	C	H	M	OLV	E	F	C	H	M	B	E	F	C	D	T	B	E	F	C	H	P
H0011	OLV	E	FF	C	H	M	OLVB	E	F	C	H	M	B	E	F	C	D	T	B	E	F	C	H	P
H0014	OLVB	E	FF	C	H	M	OLVB	E	F	C	H	M	B	E	F	C	D	M	B	E	F	C	H	P
H0017	OLVB	E	FF	C	H	M	OLVBF	E	F	C	H	M	B	E	F	C	D	M	B	E	F	C	H	P
H0019	OLVBF	E	FF	C	H	M	OLVB	E	FF	C	P	M	RB	E	F	C	H	P	GOLV	E	F	C	H	P
BJ0011	OLVB	E	FF	C	P	M	OLV	E	F	C	H	M	S	E	F	C	D	M	B	E	F	C	D	M
CS002	GOLV	R	FF	FL	H	P	OLVBF	E	FF	FL	H	M	S	R	F	C	D	M	LG	R	FF	FL	P	P
A021	OLVBF	E	FF	FL	H	T	OLV	E	FF	FL	H	M	S	RC	FF	C	D	M	S	E	F	C	D	T
CH002	OLVB	AC	FF	FL	H	M	GOLV	E	FF	FL	H	M	S	RC	FF	C	D	T	S	E	F	C	D	T
CH004	OLVB	AC	FF	FL	H	M	GOLV	E	FF	FL	H	M	B	RC	FF	C	D	M	S	E	F	C	D	T
CH005	OLVB	AC	FF	FL	H	M	OLV	E	FF	FL	H	M	S	RC	FF	C	D	M	RB	E	F	C	D	T
CH006	OLV	AC	FF	FL	H	M	OLV	E	FF	FL	H	M	B	RC	FF	C	H	P	RB	E	F	C	D	T
CH007	OLV	AC	FF	FL	H	M	OLV	E	FF	FL	H	M	S	RC	FF	C	D	M	S	E	F	C	D	T
CH008	OLV	AC	FF	FL	H	M	OLVBF	E	FF	C	H	M	RB	RC	FF	C	D	M	RB	E	F	C	D	T
CH009	OLVBF	E	FF	FL	H	P	OLV	E	F	C	D	P	B	RC	FF	FL	D	T	S	E	F	C	D	T
T006	OLVB	E	FF	FL	H	P	OLVBF	R	F	C	H	P	B	RC	F	C	D	T	B	E	FF	FL	D	T
T0012	GOLV	R	F	C	D	T	OLVBF	R	F	FL	H	P	B	RC	FF	C	D	T	GOLV	R	FF	FL	H	P
T0017	OLV	E	FF	FL	H	P	OLV	E	F	FL	H	P	LG	RC	FF	C	D	T	LG	R	F	C	P	M
N021	OLV	E	FF	FL	H	P	OLV	E	F	FL	D	T	LG	R	FF	C	H	P	LG	R	F	C	P	M
N022	OLV	E	F	FL	D	T	OLV	E	F	FL	H	T	LG	RC	FF	C	H	P	LG	E	FF	FL	H	P
V021	OLV	E	F	FL	H	T	OLV	E	F	FL	D	T	B	R	F	C	H	P	LG	R	FF	FL	P	M
V022	OLV	E	F	FL	D	T	OLV	E	FF	FL	H	P	B	RC	F	C	D	T	LG	R	FF	FL	P	M
V0212	OLV	E	FF	FL	H	P	OLV	E	FF	FL	P	P	LG	RC	FF	C	D	M	LG	R	FF	FL	P	M
V0214	OLV	E	FF	FL	P	P	OLV	E	FF	FL	H	P	LG	R	F	C	H	M	LG	R	FF	C	H	P
B022	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	F	C	D	T	B	R	FF	FL	H	P
B023	OLV	E	FF	FL	H	P	OLVBF	E	FF	FL	H	P	B	RC	F	C	D	P	LG	R	F	C	H	P
B025	OLV	E	FF	FL	H	P	OLV	E	FF	FL	D	T	B	RC	FF	C	D	T	LG	R	F	C	P	M

Table 2 continue

B026	OLV	E	FF	FL	D	T	OLV	E	FF	FL	H	P	LG	RC	FF	C	D	T	LG	E	FF	FL	D	T
B029	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	D	P
G031	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	D	P
G032	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	D	T
G035	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	H	M	LG	E	FF	FL	H	P
G036	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	P	P
G037	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	P	T
G038	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	H	M
G039	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	P	T
G0310	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	H	M	LG	E	FF	FL	P	P
G0311	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	P	T
G0312	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	H	P
G0313	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	H	P
G0315	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	H	M
G0316	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	P	M
G0317	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	D	M
G318	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	H	M
G0319	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	H	M	LG	E	FF	FL	H	T
G0321	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	D	T
G0322	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	H	T
G0323	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	H	T
G0324	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	D	P
G0325	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	D	M
G0326	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	H	M	LG	E	FF	FL	D	M
G0327	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	H	P
G0328	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	D	M
G0329	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	P	M
G0330	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	H	M
G0331	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	P	P
G0332	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	P	P
G0334	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	RC	FF	C	P	M	LG	E	FF	FL	P	P
G0336	OLV	E	FF	FL	H	P	OLV	E	F	FL	H	P	LG	RC	FF	C	D	M	RB	E	FF	FL	H	M
TA024	OLV	E	F	FL	H	T	OLVB	E	F	C	H	M	B	RC	FF	C	D	T	B	RC	FF	C	H	T
O008	OLVB	E	F	C	H	P	OLV	E	F	C	H	T	LG	RC	FF	C	D	T	B	E	FF	FL	D	T
V025	OLV	E	F	C	H	T	OLV	E	F	C	H	T	LG	RC	FF	C	D	T	RB	R	FF	FL	H	P
V024	OLV	E	F	C	H	T	OLVB	E	FF	C	P	M	RB	E	F	C	H	P	GOLV	E	F	C	H	P
TA021	OLV	E	F	FL	H	T	OLV	E	F	C	H	T	LG	RC	FF	C	D	M	B	E	FF	FL	D	T
CS001	OLVB	AC	FF	FL	H	P	OLVB	E	FF	C	H	P	S	RC	F	C	H	T	B	E	F	C	D	M
A029	OLV	E	FF	FL	H	T	OLV	E	FF	C	H	T	LG	RC	FF	C	D	M	LG	E	FF	FL	D	T
B021	OLV	R	FF	FL	H	P	OLV	R	FF	FL	H	P	S	R	F	C	D	M	B	R	F	C	H	P

Table 2 continue

V0213	OLV	E	F	FL	H	P	OLV	E	F	FL	H	P	LG	E	F	C	D	T	LG	E	FF	FL	D	T
A0210	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	P	LG	R	F	C	H	P	LG	R	FF	FL	P	P
AZ022	OLVB	E	FF	FL	H	M	OLV	E	FF	FL	P	M	LG	R	FF	C	H	P	LG	E	FF	FL	H	P
H021	OLV	E	FF	FL	H	T	OLV	E	FF	FL	P	T	B	R	FF	C	H	P	GOLV	R	FF	FL	P	M
T009	OLVB	E	FF	FL	H	M	OLVB	E	F	C	D	T	B	RC	FF	FL	D	T	S	E	F	C	P	M
T008	OLVB	E	FF	FL	H	P	OLVB	E	F	C	D	T	RB	RC	FF	FL	D	T	S	E	F	C	P	M
T001	OLVB	E	FF	FL	H	P	OLVB	E	F	C	D	T	B	RC	FF	FL	D	T	S	E	F	C	P	M
H004	OLVB	E	FF	C	H	M	OLVB	E	F	C	H	M	B	E	F	C	D	M	B	E	F	C	H	P
H0018	OLV	E	FF	C	H	M	OLV	E	FF	C	H	M	B	E	F	C	D	M	B	E	F	C	H	P
O0011	OLVB	E	F	C	H	P	OLVB	E	F	C	H	M	B	RC	FF	C	D	T	B	E	FF	C	H	P
BJ0012	GOLV	E	FF	C	P	M	GOLV	E	FF	C	P	M	GOLV	E	F	C	H	P	GOLV	E	F	C	H	P
H007	OLVB	E	FF	C	H	M	OLVB	E	FF	C	H	M	B	E	F	C	D	M	B	E	F	C	H	P
H0012	OLVB	E	FF	C	H	M	OLVB	E	FF	C	H	M	B	E	F	C	D	T	B	E	F	C	H	P
H001	OLVB	E	F	C	H	M	OLVB	E	F	C	H	M	B	E	F	C	D	M	B	E	F	C	H	P
G034	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	M	LG	E	F	C	H	P
G0300	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	FF	C	H	M	LG	E	FF	FL	H	P
G0333	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	FF	C	H	M	LG	E	F	C	H	P
G0320	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	FF	C	H	M	LG	E	F	C	H	P
MJ031	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	T	B	E	F	C	H	P
B033	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	B	R	FF	C	P	M	B	E	F	C	H	P
B0210	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	B	R	FF	C	P	P	B	E	F	C	H	P
B027	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	F	C	H	P	B	E	F	C	H	P
B024	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	LG	RC	FF	C	D	T	LG	E	FF	FL	H	P
H0119	OLVB	E	FF	C	H	P	OLV	E	FF	FL	H	M	B	E	FF	C	D	T	B	E	F	C	H	P
H0120	OLVB	E	FF	C	H	M	OLV	E	FF	FL	H	M	B	E	F	C	D	T	B	E	F	C	H	P
B028	OLV	E	FF	FL	H	P	OLVB	E	FF	FL	H	M	LG	RC	FF	C	D	T	LG	R	FF	FL	P	P
H016	OLVB	E	FF	C	H	M	OLVB	E	FF	FL	H	M	B	E	F	C	D	T	B	E	F	C	H	P
H013	OLVB	E	FF	C	H	M	OLVB	E	FF	C	H	M	B	RC	F	C	D	T	B	E	F	C	H	P
H012	OLVB	E	FF	C	H	M	OLVB	E	FF	C	H	M	B	E	F	C	D	T	B	E	F	C	H	P
H015	OLVB	E	FF	C	H	M	OLVB	E	FF	C	H	M	B	E	F	C	D	T	B	E	F	C	H	P
H0111	OLVB	E	FF	C	H	M	OLVB	E	FF	C	H	M	B	E	F	C	D	T	B	E	F	C	H	P
O0018	OLVB	E	F	C	H	P	OLVB	E	F	C	H	M	B	RC	FF	C	D	T	B	E	FF	C	H	P
CH0010	OLVB	E	FF	C	H	P	OLVB	E	FF	FL	H	M	S	RC	FF	C	H	P	S	E	F	C	D	T
C041	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	B	R	FF	C	H	T	S	E	F	C	H	P
C042	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	B	R	FF	C	H	T	S	E	F	C	H	P
C043	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	B	R	FF	C	H	T	S	E	F	C	H	P
FH041	OLV	E	FF	FL	H	P	OLV	E	FF	FL	H	M	B	R	F	C	P	T	B	E	F	C	H	P

COLOUR: OLV = olivaceous; OLVB = olivaceous black; OLVBF = olivaceous buff; GOLV = greenish olivaceous; S = salmon; B = buff; RB = rosy buff ; LG = lavender grey SUP =surface: E = even; R: ridged, RC = concentric rings EDGE: F = firm; FF = filamentous FORM: C = circle; FL = filamentous MORP = morphology of colony: H = heavy; P = tuf; D = down AM = development aerial mycelia: T = thin; M = moderate; P = plentiful

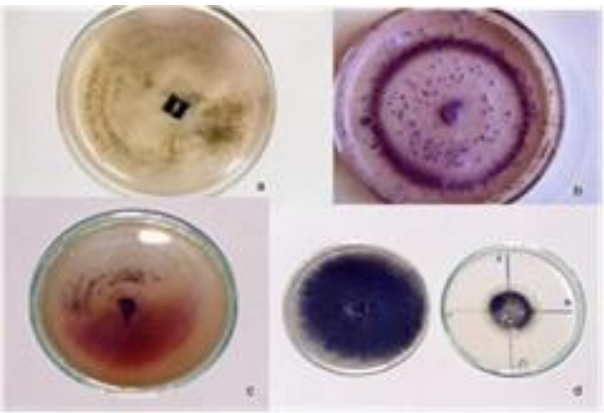

Figure 2. Colony variability of *P. tritici-repentis* isolates.
a: Isolate 9J031 cultivated under TIII; b: Isolate CHI0313 cultivated under TIII; c: Isolate BJ0011 cultivated under TIII and d: isolate BJ0011 cultivated under TII.

Table 3. ANOVA of relative growth rate of isolates of *Pyrenophora tritici-repentis*.

		Mean Square			
	DL	**TI**	**TII**	**TIII**	**TIV**
Isolates	154	0.262413***	0.382678***	0.0514711***	0.04801***
Error	155	0.0423339	0.00720581	0.00076129	0.00162903
Total	309				

Treatments: TI: 2% PDA 12 hs light-dark; TII: 2% PDA continuos dark; TIII: V8-agar 12 hs light-dark; TII: V8-agar continuos dark

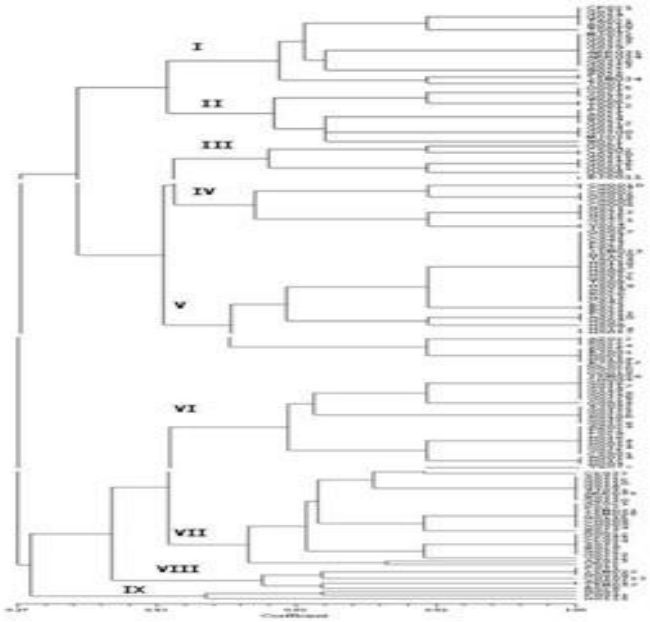

Figure 3. Dendrogram produced by UPGMA cluster analysis based on morphocultural characteristics produced by 155 isolates of P. *tritici-repentis*.

Figure 4. Tan spot symptoms on K. Chajá, K. Flecha and Baguette 10.

Table 4. ANOVA of the leaf infected area (%) of eight wheat cultivars infected with 155 isolates of *P. tritici-repentis* under greenhouse conditions.

Source of variation	Degree of freedom	Mean square
Blocks	2	6.462
Isolates	154	11.242 ***
Error	308	8.624
Cultivars	7	54.602 ***
Isolate x cultivar	1078	5.895 ***
Error	2170	3.583
Total	3719	

CV= 27.70%. ns: non significant; ***: significant at p=0.05.

three isolates, H003, H0111 and FH041, obtained from Los Hornos.

Pathogenicity test

Disease symptoms characterized by brown-black spots, chlorosis and brown-black spots with chlorosis were observed in all the set of wheat cultivars tested 72 h after inoculation. The isolates of *P. tritici-repentis* did not grouped by the reaction type on the eight wheat cultivars tested. The typical symptom observed was necrotic spots with a chlorotic halo (Figure 4). Only three isolates of *P. tritici-repentis* (CP021, AZ023 and O0014) did not produced symptoms on the set of wheat cultivars inoculated.

The analysis of variance (ANOVA) of the severity data indicated significant differences among isolates, cultivars and the interaction isolate x cultivar (Table 4). The Tukey's test revealed significant differences for the interaction isolate x cultivar for 33 isolates of the total of the *P. tritici-repents* isolates tested (Table 5).

These isolates presented 25 different interaction patterns on the set of wheat cultivars assayed. Only four patterns were shared by the isolates TA022, O0012, A021, P026 and AD031; TA021 and O0019; P022, H008 and H0011; G035 and G0325 respectively.

Table 5. Leaf infected area (%) of eight wheat cultivars with 155 isolates of *P. tritici-repentis* isolates.

Cultivar	O0017	P022	P026	S031	S032	AD031	ARR034	ARR035
KLEIN JABALI	0.107 b	0 b	0.277 b	0.887 bc	1.387 ab	0 b	0.167 b	0.553 b

Cultivar	OL022	TA022	AZ021	AZ024	O001	O0020	O0012	O0019
KLEIN JABALI	0 b	1.33 b	0 b	0 b	0 d	0 b	0 b	0 b
KLEIN CHAJA	4.66 a	8.167 a	16.00 a	3.163 a	1.773 bcd	1773 a	5.987 a	1.663 b
BUCK PREMIUM 13	0.217 b	0 b	0.66 b	0.717 ab	0 d	0 b	0.44 b	0 b
BAGUETTE 10	0.220 b	0.887 b	5.49 ab	0.107 b	1.053 bcd	0 b	0.773 b	0.883 b
ACA 801	0.053 b	0.44 b	0 b	0.773 ab	3.273 abc	0 b	0 b	0.107 b
KLEIN FLECHA	2.717 ab	0.553 b	2.48 b	1.887 ab	6.110 a	0 b	0.887 b	5.83 a
ACA 303	0.33 b	0.330 b	0.66 b	0 b	0.667 cd	2.22 a	1.22 b	0 b
KLEIN ESCORPION	0 b	0.220 b	1.32 b	0 b	3.887 ab	0 b	0 b	0 b
KLEIN CHAJA	5.993 a	0.833 b	3.943 a	4.497 a	5 a	4.05 a	3.660 a	5.830 a
BUCK PREMIUM 13	0.387 b	0 b	0.387 b	0.110 c	0.277 b	0 b	0.220 b	1.643 ab
BAGUETTE 10	2.11 a	5.443 a	0.387 b	0.663 bc	1.887 ab	0.83 b	1.050 ab	0 b
ACA 801	0.163 b	1.107 ab	0.663 b	0.883 bc	0.663 ab	0 b	0.55 ab	0 b
KLEIN FLECHA	1.773 a	0.997 b	0.667 b	2.773 ab	1.720 ab	0.883 b	0.11 b	0 b
ACA 303	0.387 b	0 b	0 b	0.107 c	0.44 b	0.610 b	0 b	2.163 ab
KLEIN ESCORPION	2.500 a	0 b	0 b	0 c	0 b	0.107 b	0.443 b	0 b

Cultivar	25M033	H003	H008	H0011	A021	CH004	B022	G035
KLEIN JABALI	0.22 bc	0.053 b	0 b	0 b	0.33 b	0.053 b	0 b	1.833 b
KLEIN CHAJA	4.107 a	0.22 b	1.443 b	0.163 b	5.553 a	1.333 ab	1.833 ab	0.387 b
BUCK PREMIUM 13	0.053 c	0.553 ab	0.110 b	0.720 b	0.167 b	0.167 ab	0 b	1.110 b
BAGUETTE 10	0.667 bc	0 b	7 a	5.167 a	0.167 b	0.053 b	0.333 b	6.053 a
ACA 801	1.22 abc	0 b	2.110 ab	1.110 ab	1.167 b	0 b	0 b	0.497 b
KLEIN FLECHA	2.940 ab	0.553 ab	0 b	0 b	0.443 b	0 b	0 b	0 b
ACA 303	0.22 bc	4.720 a	0.053 b	0 b	0.667 b	0 b	5.167 a	0.333 b
KLEIN ESCORPION	0 c	1.110 ab	1.773 b	0.610 b	0.167 b	2.887 a	1.387 ab	0 b

Cultivar	G0325	TA021	A0210	H004	G034	G0333	H0119	H012	H015
KLEIN JABALI	1.833 b	0.277 b	5.11 a	0.387 ab	0.330 b	0.443 b	0.163 b	2.383 a	0.440 b
KLEIN CHAJA	0.387 b	0 b	0.387 b	1.330 ab	0.607 ab	0.273 b	0 b	0.053 ab	0.217 b
BUCK PREMIUM 13	1.110 b	0 b	0.720 b	3.163 a	0.053 b	1.387 ab	2.277 ab	1.553 ab	0.053 b
BAGUETTE 10	6.053 a	0.11 b	0.163 b	1.887 ab	1.050 ab	0.940 ab	5 a	0.053 ab	1.277 ab
ACA 801	0.497 b	0 b	1.383 b	1.387 ab	4.773 a	1.667 ab	0.443 b	0.220 ab	1 ab
KLEIN FLECHA	0 b	11.053 a	0.887 b	0.443 ab	1.330 ab	0 b	0.053 b	0.110 ab	4.163 a
ACA 303	0.333 b	0.940 b	0.443 b	0.163 b	0.220 b	0.330 b	1.330 ab	0 b	2.330 ab
KLEIN ESCORPION	0 b	0.553 b	0.333 b	0.663 ab	0.220 b	5.053 a	0.940 ab	0.500 ab	0 b

The means followed the same letters it is no significant at p=0.05

DISCUSSION

It is recognized that *P. tritici-repentis* presents great variability (de Wolf et al., 1998). Several studies to detect variability include different tools, the most of them used pathogenic and genetic characterization (PCR techniques) (Ali et al., 2010; Andrie et al., 2007; Friesen et al., 2005; Leisová et al., 2008; Lepoint et al., 2010; Litcher et al., 2002; Mehta et al., 2004; Moreno et al., 2009; Pujol Vieira dos Santos et al., 2002; Singh and Hughes, 2006). A lesser extent of research focuses on characterization based on morphological and biochemical aspects (Diaz de Ackermann 1987; Gilchrist et al., 1984; Hosford, 1971; Hunger and Brown, 1987, Moreno et al., 2008a; Odvody and Boosalis, 1982; Pujol Vieira dos Santos et al., 2002). It has been noted in these studies that isolates of *P. tritici-repentis* vary greatly.

The isolates developed a thick dark and filamentous mycelia when were incubated in PDA. Similar results were observed by Hosford (1971) and Glichrist et al. (1982). Mc Donald (1967), Frazon et al. (2001) and observed variability in mycelium colour in several *Drechslera teres* isolates cultivated with PDA. Matsumura (1991), Valim-Labres et al. (1997) and Oliveira et al. (1998) found variability in mycelium colour and colony growth in *Bipolaris sorokinianna* grown on PDA. Some isolates of *P. tritici-repentis* showed a pink range colony colour. These results are in correspondence with those observed by Hosford, (1971). Hunger and Brown, (1987) observed this coloration from colonies developed from ascospores of *P. tritici-repentis*. Diaz de Ackermann (1987) also observed isolates of *P. tritici-repentis* that did no present the same colony colour. However, Gilchrist et al. (1984) observed that colonies of *P. tritici-repentis* maintained the colony colour. In our study we identified different of colony growth. Similar results were observed by Christensen and Graham (1934), which showed differences in the colony growth rate. Whemeyer (1954), detected significant differences among isolates respect to relative growth of colonies of *P. tritici-repentis*.

The isolates incubated on agar-V8 developed conidiophores and conidia more profusely when they were incubated under cycle 12 h light-12 h dark. These confirm the observations made by others authors (Diaz de Ackermann, 1987; Hosford, 1971; Hunger and Brown, 1987).

The results obtained for conidial development in this study confirm previous observations realized by several authors (Elias 1987; Gilchrist et al., 1984; Luz and Hosford, 1980; Odvody and Boosalis, 1982). *P. tritici-repentis* cultivated in continuous dark did not produce conidiophores so no conidia developed. Those isolates incubated on agar-V8 produced a high number of conidia mm^{-2} and showed a colony colour lavender grey and a colony diameter of 0.50-1.50 cm. The different production of conidia between TIII and TIV suggested that 24 h of light are not sufficient to achieve high levels of sporulation when isolates were incubated for seven days under darkness.

The dendrogram showed distinct groups of morphotypes (44) of *P. tritici-repentis* with the highest similarity coefficient. These results suggest a high diversity among the *P. tritici-repentis* isolates under culture conditions. According this cluster analysis an association between the morphocultural variability and the geographic origin from which the isolates were obtained could not be established. Each of the nine subcluster contained isolates originated from different localities and wheat cultivars. The high variability under culture conditions might be attributed to the high frequency of hyphal anastomosys observed in the asexual stage. The hyphal anastomosys is in relation with the heterokariosis. This affects the variability of fungi, the virulence and the pathogenic characteristics of the pathogens (Agrios 2004). In agreement with others authors (Burguess et al 1995; Teixeira et al 2004), the morphocultural markers are highly variables and very dependent of the *in vitro* culture conditions and the intrinsic variability of the pathogen. In this way, the analysis of similarity based in the morphocultural markers did not help in finding a correlation between variability morphocultural of isolates and geographic origin.

In our study, the first symptoms of the disease were observed at 72 h post-inoculation with an HR of 50-85%. These results confirm previous observations of Mehta et al (2004). However, Sah and Fehrman (1992) observed the first symptoms latter (at 92 h) and with a HR of 65-95%. The analysis of variance (ANOVA) of percent severity indicated significant differences in the interaction isolate x cultivar. According to Van der Plank (1978, 1984) specificity in host-pathogen relationships is often indicated by significant isolate x cultivar interaction in the analysis of variance of an experiment, where a number of pathogen isolates are tested in all possible combinations on a set of host genotypes. Non-specificity is identified by a lack of such interaction. In this study we observed significant interaction isolate x cultivar for 16% of the population of *P. tritici-repentis* tested. The level of severity observed did not allow differentiate between susceptible and moderately resistant wheat cultivars. Some reasons could explain the low degree of severity observed. Among these, the early evaluation time, the inoculum concentration used, the greenhouse conditions, and the susceptibility of cultivars selected cold be influenced. Differences in the virulence of isolates of *P. tritici-repentis* were reported in previous works (Ali and Francl 2001, 2003; Ali et al., 1990; Gamba et al., 1998; Krupinsky 1987, 1992a,b; Luz and Hosford 1980; Lamari and Bernier 1989a,b; Lamari and Gilbert 1998; Lamari et al., 1995, 1998; Moreno et al., 2008 b; Sah and Ferhmann, 1992; Schilder and Bergstrom, 1990). Some results considered that the number of significant interactions was low so the isolates are different in their aggressiveness and non in their virulence (Mehta et al.,

2004). Others author observed moderate level of physiological specialization (Schilder and Bergstrom, 1990). Lamari and Bernier (1989b) tested 92 isolates on 11 cultivars of wheat and reported significant interaction isolates x cultivar.

According to Chen et al. (1993) the population structure of a pathogen is influenced by the population of the host. The production of races is one the most important characteristics where the pathogen express their variability. The crossing-over, mutations, heterokariosis and the adaptation are some of the factors that affect the variability of the pathogens (Agrios, 2004). The races can differ in level of growth, colony colour and others characteristics under in vitro conditions as nutrition, temperature requirement, enzymatic activity, tolerance to pH, fungicides or in the pathogenic behaviour among others character (Wolf and Wolf, 1947).

Consideration of variation in virulence in the *P. tritici-repentis* population is essential to understand the pathogen genetics of tan spot. Knowledge of the pathogen population helps in the development of a successful disease management program, particularly resistant cultivars, effective fungicides and biological control agents. In particular, all wheat lines should be screened with all races prevalent in the region prior to their commercialization.

In to developed durable resistant cultivars to control plant diseases, knowledge of the diversity of plant pathogens is essential (Ali and Francl, 2002). *P. tritici-repentis* is a homotallicus fungus that readily produces the sexual stage on wheat stubble under field conditions in Argentina, giving the fungal population the opportunity for adaptation by sexual recombination. Therefore, these may have a part to play in explaining the high level of morphocultural and pathogenic differences found among the *P. tritici-repentis* isolates used in our study.

The work presented here shows valuable information on variability of the argentinian population of *P. tritici-repentis* unknown until now. In accordance, a high variability with biochemical and DNA techniques (Moreno et al., 2008 a,b), and population structure in races (Moreno et al., unpublished data) was detected after that. This information is necessary for the breeding of wheat in Argentina, as an important tool for the identification of resistance genetic of the pathogen.

ACKNOWLEDGEMENTS

This work was supported by grants provided by the Universidad Nacional de La Plata, PRH 32-PICT 149 and Proyecto Regional Trigo-PROCISUR. We thanks, Dr Stenglein for the reading of the manuscript.

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