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Pyrenophora tritici-repentis, Causal Agent of Tan Spot: A Review of Intraspecific Genetic Diversity

M. V. Moreno[1,2,3], S. A. Stenglein[1,2,3] and A. E. Perelló[1,4]
[1]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)*
[2]*Laboratorio de Biología Funcional y Biotecnología*
(BIOLAB)-CEBB-CONICET, Facultad de Agronomía de Azul
Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA)
[3]*Microbiología Agrícola, Facultad de Agronomía, UNCPBA*
[4]*Centro de Investigaciones de Fitopatología (CIDEFI)*
Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata
Argentina

1. Introduction

1.1 Currents status of tan spot

In some countries where the wheat is cultivated, the biological adversities are led by foliar disease. These diseases have emerged as a serious problem in many areas where the wheat is the principal crop. In the last few years, minimum tillage has been considered advantageous to soil conservation, but it leads to a loss of available nutrients and a potential increase in necrotic pathogens whose saprophytic stage lives in the straw of the crop (Annone, 1985). Establishment of the crop under this management can be affected by pathogens of this type. Leaf spotting diseases can be caused by one or a combination of leaf spotting pathogens (Table 1). Leaf spotting diseases affect wheat grown reduce the photosynthetic area of leaves resulting in reduced grain filling and lower yields; particularly when the top two leaves (penultimate and flag leaves) are severely infected. The most of these diseases are similar in host symptomatology, disease cycle, life cycles of pathogens and types of damage induced.

Whitin of these pathogens the Ascomycota fungus, *Pyrenophora tritici-repentis* (Ptr) (Died.) Drechs. It is a facultative pathogen whose asexual stage is *Drechslera tritici-repentis* (Dtr) (Died.). This pathogen is the causal agent of tan spot of wheat.

The effects of tan spot on wheat yields have been reported from 1974 by Hosford & Busch. Several authors had observed severe attacks of *P. tritici-repentis* (Adee & Pfender, 1989; Annone 1994; Fernandez et al., 1998; Galich & Galich 1994; Kohli, 1992; Metha & Gaudencio, 1991; Rees et al., 1982; Rees and Platz, 1983; Sharp et al., 1976). The tan spot has induced losses that reached from 20 % to 70%of yield and it is very destructive on durum, winter and spring wheat (Lamari & Bernier 1989a, b; Misra & Singh 1972). Tan spot reduces total yield,

kernel weight (Shabeer & Bockus 1988; Schilder & Bergstrom 1990), number of grains per head (Schilder & Bergstrom 1990), total biomass (Kremer & Hoffmann 1992), and/or grain quality because of red-smudge symptoms (Fernandez et al., 1994).

Teleomorph	Anamorph	Disease
Pyrenophora tritici-repentis (Died)	*Drechslera tritici-repentis* (Died)	Tan Spot
Mycosphaerella graminicola (Fuckel) J. Schrot. in Cohn	*Septoria tritici* (Roberge in Desmaz.)	Leaf Blotch
Phaeosphaeria nodorum (E. Müller)	*Stagonospora nodorum* [Berk.] Castellani & E.G. Germano)	Stagonospora Nodorum Blotch
Cochliobolus sativus (S. Ito & Kurib.) Drechsler ex Dastur	*Bipolaris sorokiniana*	Spot Blotch

Table 1. Leaf spotting complex of wheat

The increasing inoculum of this pathogen has been attributed to use of conservation tillage systems, shorter crop rotations, continuous wheat cultivation and use of susceptible cultivars. The sexual phase survives in wheat stubble (Rees and Platz, 1980). Currently, we consider the tan spot disease has shown the highest growth in crops under zero-tillage. Interestingly, collateral hosts of *P. tritici-repentis* could play an important role as a source of primary inoculum between growing seasons, as a source of genetic variation, and as a reservoir of a fungal population genetically different from that prevalent on wheat (de Wolf et al., 1998).

Tan spot has been found in several countries worldwide like Australia, Argentina, Asia, Belgium, Brazil, Bolivia, Canada, Colombia, Czech Republic, Denmark, Ecuador, France, Hungary, Kenya; Pakistan, Paraguay, Peru, Poland, United States of America, Ucrania and Uruguay (Ali & Francl, 2001_a; Annone, 1985, Conners, 1939; Dubin, 1983; Duveiller et al., 1996; Gilchrist et al., 1984; Hosford, 1981 Kohli et al., 1992; Lamari et al., 2005; Linhares & da Luz, 1994; Loughman et al., 1998; Maraite et al., 2006; Mehta et al., 2004; Moreno et al., 2008; Postnifova & Khasanov, 1998; Sarova et al., 2002; Tekauz, 1976; Sim, and Willis, 1982; Watkins, et al., 1978; Zhanarbekova et al., 2007).

The control of this disease is complex and forms part of the concept of integrated disease management, that is, a combination of chemical , cultural, genetic, and sometimes biological controls (Annone, 2005, 2006; Bockus et al., 1992; Carmona, 2003; Duckez & Jones-Flory, 1994; Hosford & Busch, 1974; Li & Sutton, 1995; Loughman et al., 1998; Luz et al., 1998; Perelló et al., 2003, 2006; Pfender et al., 1989; Simón, 2006; Simón et al 2011; Stover et al., 1996; Sutton & Roke, 1996; Zadoks & Schein, 1979).

In the last 30 years, some investigations in the complex wheat-*P. tritici-repentis* have been deeply developed. In 1989a,b, Lamari and collaborators suggested the presence of races of *P. tritici-repentis* based in the interaction with different cultivars and lines of wheat. Before, some researches had already mentioned the existence of physiological specialization of this pathogen. The fungus produces two distinct symptoms, necrotic (nec) and chlorosis (chl), on susceptible wheat genotypes. These symptoms are the result of the production of multiple host-selective toxins (HSTs).

1.2 The pathogen

*Pyrenophora tritici-repentis (*Died.) Drechs., is the causal agent of tan spot of wheat leaf. It is a facultative parasite fungus (saprotroph) of Phylum Ascomycota, Class Loculoascomycetes, whose anamorph corresponds to *Drechslera tritici-repentis* (Died.) Shoeman., imperfect fungus of the class Hyphomycetes.

It was first isolated from *Agropyron repens* in Germany and determined as *Pleospora trichostoma* by Diecke. In 1928 was isolated from wheat Nisikado, who called *Helminthosporium tritici-repentis* (= *Drechslera tritici-repentis*) (Hosford, 1981).

1.2.1 Taxonomy

Teleomorph:

Pyrenophora tritici-repentis (Died.) Drechs, J. Agr. Research, 24:667. 1923.

_ *Pleospora tritici-repentis* (Died.), Centr. Bakt. Parasitenk, Abt. 2, 11:56. 1903.

_ *Pleospora trichostoma* (Fr.) Wint. f. *sp tritici-repentis* Died., Centr. Bakt. Parasitenk., Abt. 2, 9:329. 1902.

= *Pyrenophora tritici-vulgaris* Dickson, Diseases of Field Crop, ed. 2, Mc Graw-Hill, New York, 1956. P. 247. nom. nud.

Anamorph:

Drechslera tritici-repentis (Died.) Shoem. Canadian Journal of Botany, 37:880, 1959.

_ *Helminthosporium tritici-repentis* (Died.) Died.; Centr. Bakt, Parasitenk, Abt. 2, 11:56.1903.

_ *Helminthosporium graminearum.* Rob. Ex Schlecht. f. sp. t*ritici-repentis* Died, Centr. Bakt. Parasitenk, Abt. 2, 9:329. 1902.

= *Drechslera tritici-vulgaris* (Nisikado), Ito, Proc. Imp. Acad., Tokio, 6-355, 1930.

_ *Helminthosporium tritici-vulgaris* Nisikado, Ann. Phytopath. Soc. Japan, 2:96. 1928.

1.2.2 Morphological characteristics

The ascocarp (protoseudothecia) of *P. tritici-repentis* is dark brown, spherical to subspherical, smooth, from 400 to 500 µ of diameter. The beak bears a number of dark-brown sterile setae near the ostiole. These setaes has from 80 to 120 µ of lenght and 9 µ of wide. The asci measure usually from 170 to 215 by 43 to 50 µ. The ascospores are brownish colored and measuring usually 47-65 µ of length x 20-26 µ of wide. The ascospores are biseriate and uniseriate in the middle towards the apex respectively (Figure 1a) (Ellis & Waller, 1976).

The 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 (Figure 1b). (Ellis & Waller, 1976).

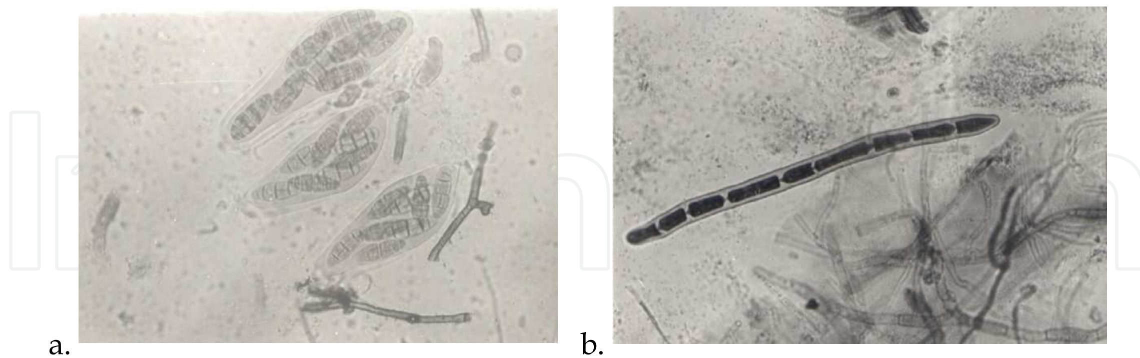

Fig. 1. a: Asci with ascospores b: conidia of *P. tritici-repentis* (=*D. tritici-repentis*) (Moreno 2007)

1.3 Disease cycle

The dispersal and the infection development by *Pyrenophora tritici-repentis* are produce among 10º to 30º C and moisture among 6hs to 48hs (Hosford et al., 1987; Larez et al., 1986; Sah, 1994). These conditions are favorable for the yearly occurrence of tan spot and to distinguish it from other disease, white head that depended of the environmental conditions (Carmona, 2003).

The disease cycle of tan spot (Figure 2) provides a convenient framework to explain the disease progress. The rate of progression through the disease cycle depends upon host, temporal and environmental components of the pathosystem (De Wolf et al., 1998).

The seeds, the straw and collateral hosts are the principal source of inoculum of tan spot. The primary inoculum wade through the wheat areas for long distances and it is introduced in new areas by the seeds. In the seed the pathogen lives in the pericarpio as mycelium and the transmission to plant rest is not systemic (Schilder & Bergstron, 1994). Another source of primary inoculum is the straw of wheat. Several authors considered the straw as the principal source of inoculum (Rees & Platz, 1980). Infested residue usually results in significant disease severity at flag leaf emergence and later growth stages due to secondary infections (McFadden, 1991; McFadden & Harding, 1989; Wright & Sutton, 1990). Collateral hosts could be inoculum source between growing seasons (de Wolf *et al.*, 1998). The tan spot fungus has been reported on many grass species from different parts of the world among these *Agropyron* sp., *Alopecurus arundinaceus*, *Andropogon gerardi*, *Avena fatua*, *A. sativa*, *Bromus inermis*, *Dactilys glomerata*, *Echinochloa* sp., *Elymus innovatus*, *Lolium perenne*, *Phalaris arundinaceae*, *Poa* sp. y *Secale cereale* (Ali & Francl, 2002_b; Andersen, 1955; Conners, 1939; Dennis and Wakefield, 1946; Dickson, 1956; Diedicke, 1902; Drechsler, 1923; Farr et al., 1989; Hosford, 1971; Howard & Morral, 1975; Krupinsky, 1992_c; Shoemaker, 1962; Sprague, 1950).

1.4 Host-parasite interactions: Symptomatology of tan spot

On susceptible wheat leaves, *P. tritici-repentis* produces characteristic oval to diamond-shaped lesions. However, newly formed tan spot lesions cannot be separated reliably from those caused by other necrotrophic pathogens. Later, lesions elongate and develop a tan

color with a chlorotic halo and a small dark brown infection site. Chlorotic areas tend to coalesce on heavily infected leaves, especially on young plants, a symptoms which led to the disease name "yellow leaf spot" (Figure 3). On resistant and partially resistant wheat, lesions size is reduced and chlorosis and necrosis may be absent (de Wolf et al., 1998). Lamari & Bernier (1989b) identified two different types of symptoms produced by the pathogen, tan necrosis and extensive chlorosis.

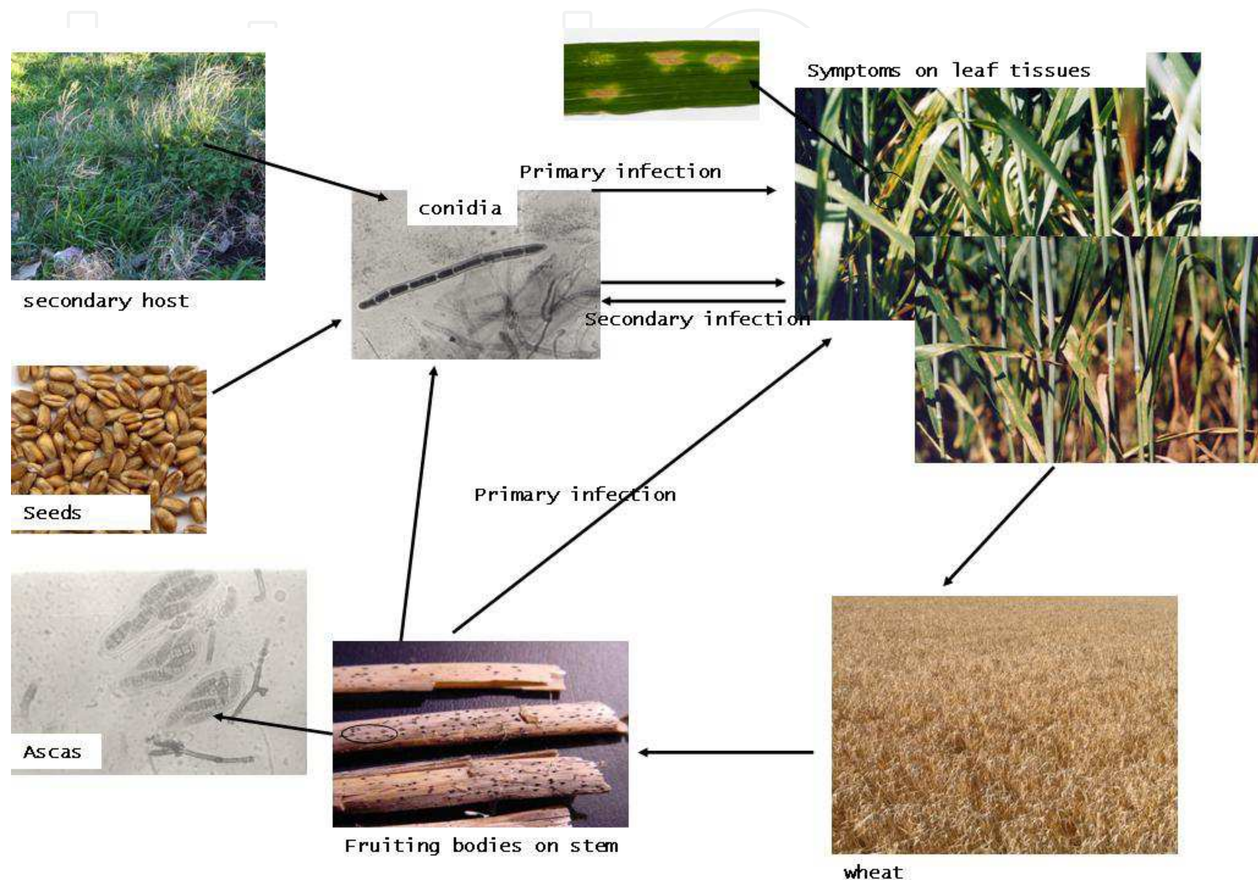

Fig. 2. Disease cycle of tan spot (Moreno & Perelló 2010)

P. tritici-repentis can also infect wheat seed during the grain filling period (Schilder & Bergstrom, 1994). This disorder is called red smudge because infected seed has a reddish discoloration (Valder, 1954).

Fig. 3. Symptoms of tan spot (Moreno & Perelló 2010)

2. The importance of host-selective toxins (HSTs) produced by *Pyrenophora tritici-repentis*

It is known that in the relation gene-for-gene, a pathogen carries a dominant Avr gene and its action result in the induction of the resistance response in a plant that carries the cognate resistance gene (Flor, 1971). Some plant pathogenic fungi produce agents of compatibility, namely host-selective toxins (HSTs). "The genetic of the *P. tritici-repentis*-wheat interaction is reminiscent of gene-for-gene type interactions" (Manning et al., 2004). In the case of *P. tritici-repentis*-wheat the interaction between Tox A and its corresponding host genes results not in resistance but susceptibility (Lamari et al., 2003).

2.1 The host selective toxin produced by *Pyrenophora tritici-repentis*

In recent decades due a great interest on the tan spot of wheat, several research groups worked about composition and identification of host-selective toxin produce by *P. tritici-repentis.* Why is important the characterization of these toxins?. The HSTs are responsible for disease symptoms on susceptible wheat cultivars and lines. At the present, three toxins have been described (Ballance et al., 1989; Ciuffetti et al., 1998; Effertz et al 2002; Martinez et al 2001; 2004; Tomas et al., 1990; Touri et al., 1995; Strelkov et al 1999, 2006). It is commonly accepted that the names of toxin are Ptr Tox A, Ptr Tox B and Ptr Tox C (Ciuffetti et al 1998). The Tox A has been described by several laboratories (Ballance et al., 1989; Ciuffetti et al., 1998; Tomas et al., 1990; Touri et al., 1995). Tomás and Bockus (1987), cited for first time the possible presence of a necrosis-inducing toxin. Five research groups have worked on the isolation and characterization of the Tox A using isolates obtained from Kansas, United State of America (Pt1c) (Ciuffetti et al., 1997; Tomás et al., 1990; Touri et al., 1995) and Canadá (82-124) (Ballance et al., 1989; Zhang et al., 1997). Only three groups have isolated and cloned the gene encoding for Tox A. Ciuffetti and colleagues (1997) used the isolate Pt1c; Zhang et al., (1997) and Ballance et al., (1989) worked about the isolate 82-124. The comparison of these sequences shows differences only for one base. Different studies corroborated the association between toxin production and necrotic symptoms, Lamari and Bernier (1989b), enumerated some points in a) toxin sensitivity correlated with pathogen-induced necrosis in test involving different cultivars, b) sensitivity to the toxin and development of necrosis in response to fungal infection were controlled by a single dominant gene, in all crosses made, c) the isolates nec+ induced necrosis on susceptibles cultivars or lines of wheat, d) those isolates that non produce necrotic symptoms on susceptibles plants did not produce Ptr Tox A *in vitro*. Additionally, Ciuffetti and colleagues (1997), demonstrated that Ptr Tox A functions are the factor of pathogenicity in the interaction wheat- *P. tritici-repentis*, through the transformation of non-pathogenic isolates of *P. tritici-repentis* with the *Tox* A gen. The toxin was shown to be a protein with a weight of a 13.2 kDa and contains a N-terminal pyroglutamate (Ballance et al., 1989; Tomas et al., 1990; Tuori et al., 1995, 2000, Zhang et al., 1997). This toxin is produced by race 1 and 2 (Lamari & Bernier 1989b). Lamari et al., (2003) showed that the isolates classified as race 7 and 8 also synthetized Ptr Tox A.

The pathogen *P. tritici-repentis* is also capable to produce one toxin that induced chlorosis in sensitive lines. This toxin is named Ptr Tox B (Ciuffetti et al., 1998), and was initially identified in culture filtrate by Orolaza et al., (1995). Previouslly, Brown and Hunger (1993), reported a phytotoxin that induced chlorosis in susceptible wheat genotypes. In the same work was

determined that this toxin to has a molecular mass between 800 and 1800 kDa. Orolaza et al., (1995) reported isolates obtained from Algerian that produced chlorosis and these isolates were classified as race 5 (Alg. 3-24). Strelkov et al., (1999) purified and characterized this toxin as a small protein (6.61kDa). Ptr Tox B is encoded by a multiple-copy gene, *Tox B*, comprising a 261-bp open reading frame (ORF) (Lamari et al., 2003; Martinez et al., 2001; Strelkov et al., 2002, Strelkov & Lamari, 2003). The races 7 and 8 produced Ptr Tox B inducing chlorosis on the cultivar Katepwa (Lamari et al., 2003; Strelkov et al., 2002).

Several studies predicted the existence of one third toxin (Gamba et al., 1998; Lamari & Bernier, 1991b), named Tox C. This toxin also produced chlorosis but in different cultivars than Ptr Tox B did. Effertz et al., (2002), proved its production in culture from the isolate of race 1 and it was partially purified. The production of this toxin by isolates classified as races 3, 6 and 8 was proved by several researches (Gamba et al., 1998; Lamari & Bernier, 1991; Lamari et al., 2003; Strelkov et al., 2002;). This toxin is not proteinaceous in the nature, but rather seems to be a nonionic, polar, low molecular mass molecule (Effertz et al., 2002).

At the present, Pandelova and Ciuffetti (2005) suggested that the necrotic symptoms produced by the isolates SO3 are caused by a previously undescribed proteinaceous. They have a tentative name for this toxin Ptr Tox D, in agree with the standard nomenclature published by Ciuffetti et al., (1998).

In summary, the HSTs produced by different isolates of *P. tritici-repentis* are Ptr Tox A, Ptr Tox B (and its homologs), Ptr Tox C, and a potential Ptr Tox D. At the present the studies that combined phenotypic and genotypic determination of races of *P. tritici-repentis* suggested the presence of new races of *P. tritici-repentis* that potentially produce new toxic components (Ali et al., 2010; Andrie et al., 2007; Moreno et al., (unpublished data)).

2.2 Physiological races of *Pyrenophora tritici-repentis*

The ability of an organism to produce disease can be evaluated as pathogenicity or virulence. These terms can differenciate because the pathogenicity is a general attribute of species and virulence is an attribute reserved for a particular isolate of a pathogen in relation of particular host genotype (Day, 1960). Other option according to Van der Plank (1978, 1984), considers that 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. Variation in virulence in the population of this pathogen is essential in understanding the interaction of the genomes involved in tan spot. Studies of the diversity of virulence within a pathogen population should help in the development of a successful disease management program, particularly resistant cultivars.

Basing in the host-pathogen interaction and in the reaction type on cultivars and lines of wheat, the complex wheat- *P. tritici-repentis* has been classified as a complex system for the determination of physiological specialization of the causal agent of tan spot (Lamari & Bernier, 1989a,b). Before the proposed of Lamari and Bernier (1989a), several works showed differences on virulence for *P. tritici-repentis* isolates (Cox & Hosford, 1987; Diaz de Ackermann 1987; Gilchrist et al., 1984; Krupinsky 1987, 1992 a,b; Luz & Hosford 1980; Misra & Singh 1972; Nagle et al., 1982;). In 1971, Hosford observed differences between the

reaction types on wheat cultivars produced by isolates of *P.tritici-repentis*. Misra and Singh (1972) tested isolates originated from India and detected significant differences in virulence, based on lesion size. Similar results were observed by Glichrist et al., (1984) when they tested isolates collected from Mexico on the wheat cultivar Morocco. Luz and Hosford (1980) grouped the isolates tested into 12 races based on statistical mean separation. However, Díaz de Ackermann et al., (1988) did not find differences in virulence among the isolates tested by Luz and Hosford (1980). Hunger and Brown (1987) tested nine isolates originated from USA these isolates showed significant differences on the susceptible cultivar TAM 105. Krupinsky (1987) showed differences in length lesion and percentage of severity, among isolates of *P. tritici-repentis* obtained from *Bromus inermis*.

Basing in the reaction type produced by 92 isolates of *P. tritici-repentis* obtained from Canada on 11 wheat cultivars, Lamari and Bernier (1989a) reported specific interactions between cultivars and isolates, indicating that these isolates differed in virulence. In this work they proposed the presence of three pathotypes. Hosford et al., (1990) suggested the presence of extensive chlorosis that could fit the chlorotic symptoms reported by Lamari and Bernier (1989a). Schilder and Bergstrom (1990) tested 70 isolates obtained from Canada on 12 wheat cultivars and detected significant differences among the interaction isolate x cultivar. Similar results were reported by Sah and Fehrmann (1992) for isolates origin from Brazil, Germany, India, Nepal and USA. However, Ali and Buchneau (1992) observed physiological specialization based in the reaction type for isolates obtained from USA. Mehta et al., (2004) tested 40 isolates obtained from Parana (Brazil) on six cultivars of wheat, and observed low interaction for isolate x cultivar. In 2007, Moreno detected significant differences isolate x cultivar for isolates of *P. tritici-repentis* obtained from different grown wheat areas in Argentina.

Actually, the worldwide knowledge indicates the existence of 10 races for *P. tritici-repentis* evaluated on a set international of wheat cultivars (Ali & Francl, $2001_{a,b}$, 2002_{b}; Lamari & Bernier, $1989_{a,b}$; Lamari & Gilbert 1998; Lamari et al., 1995, 1998, 2003, 2005).

2.3 Determination and identification of *Pyrenophora tritici-repentis* races

Lamari and Bernier (1989b), suggested that individual isolates of *P.tritici-repentis* induced individual symptoms on specific cultivar/lines of wheat. In this work, they made the following rating system base on lesion type recognized previously by Hosford (1971) and Gilchrist et al., (1984). The rating systems consisted in: 1= Small dark brown to black spots without any surrounding chlorosis or tan necrosis (resistant); 2= Small dark brown to black spots with very little chlorosis or tan necrosis (moderately resistant); 3= Small dark brown to black spots completely surrounded by a distinct chlorotic or tan necrosic ring, lesion generally not coalescing (moderately resistant to moderately susceptible); 4= Small dark brown or blacks spot completely surrounded with chlorotic or tan necrotic zones, some of the lesions coalescing (moderately susceptible); 5= The dark brown or black centres may or may not be distinguishable, most lesions consist of coalescing chlorotic or tan necrotic zones (susceptible). In other work (Lamari and Bernier 1989a), they taken the same rating system and suggested the presence of four pathotypes based in the results obtained according with specific interactions between wheat cultivars and individual isolates of *P. tritici-repentis* as follow: Pathotype 1=tan necrosis or extensive chlorosis on differential cultivars (nec+ chl+); pathotype 2= only tan necrosis (nec+ chl-); pathotype 3= only extensive chlorosis (nec- chl+)

and pathotype 4= avirulent (nec- chl-). Lamari et al., (1995) suggested the incorporation of a new pathotype that corresponding with the isolates obtained from Algerian. In this work they proposed the pathotype/race classification of *P. tritici-repentis*.

The history of determination of races of *P. tritici-repentis* has been well documented (Ali et al., 1990; Ali & Francl, 2002a,b, 2003; Lamari & Bernier 1989a,b; Lamari et al., 1995, 1998; Strelkov et al., 2002). It is commonly accepted the existence of eight races that it have been characterized on three effective host differential of wheat (Lamari et al., 2003). Although, in some studies the authors proposed the existence of two races more (Ali & Francl, $2002_{a,b}$).

In summary, the races 1, 2, 3, 4 and 5 of *P. tritici-repentis* corresponds with those determined by Lamari et al., (1995). The races 1 and 2 are predominant in North America (Ali & Francl, 2003). The greater part of isolates identified as race 5, are originated from North Africa, North America and Algerian (Ali et al., 1990; Ali & Francl, 2003; Lamari et al., 1995, 1998; Strelkov et al., 2002). The races 6, 7 and 8 were identified from collections originated from Azerbaïdjan (Caucasus), Syria, Turkey and South America (Ali & Francl, 2002_{a}; Lamari et al., 2003; Strelkov et al., 2002). By other hand, the races 9 and 10 were identified from isolates originated from South America (Ali & Francl, $2002_{a,b}$). The results of these studies suggested that the variation in the virulence of population of *P. tritici-repentis*, can be detected through quantitative and/or qualitative rating scales.

The race characterization based only on phenotypic features could carry some mistakes. Andrie et al., (2007), proposed minimized these errors using a combination of phenotypic and genotipic characterization for *P. tritici-repentis* race identification.

At the present, the genotypic characterization of *P. tritici-repentis* population is wide due the incorporation of molecular tools and massive use of Polymerasa Chain Reaction (PCR). Moreover, the availability of sequences of *Tox* A, *Tox* B and *tox* b gene sequences, allowed by many researchers as Andrie et al., (2007) designed a multiplex PCR races of *P. tritici-repentis*. Its known that the virulence patterns of *P. tritici-repentis* are based on a toxin production and each compatible interaction between an isolate and its corresponding susceptible host cultivar. In this way, we considered the race structure proposed by Andrie et al., (2007), which was based in different studies (Lamari et al., 1995; 2003; Strelkov et al., 2002) (Table 2).

Races	**Glenlea**	**Katepwa**	**6B662**	**6B365**	**Salomouni**	**M3**
1	N (Tox A)	N (Tox A)	R	Cl (Tox C)	R	R
2	N (Tox A)	N (Tox A)	R	R	R	R
3	R	R	R	Cl (Tox C)	R	R
4	R	R	R	R	R	R
5	R	Cl (Tox B)	Cl (Tox B)	R	R	R
6	R	Cl (Tox B)	Cl (Tox B)	Cl (Tox C)	R	R
7	N (Tox A)	N (Tox A) Cl (Tox B)	Cl (Tox B)	R	R	R
8	N (Tox A)	N (Tox A) Cl (Tox B)	Cl (Tox B)	Cl (Tox C)	R	R

References: N= necrosis. C= chlorosis, R= resistance, Tox A= presence fragment of *Tox A* gene, Tox B= presence fragment of *Tox B* gene, Tox C= presence of Tox C as evidenced by inoculation.

Table 2. *Pyrenophora tritici-repentis* pathotype/race classification according Andrie et al., (2007).

3. Genetic variability of plant pathogenic fungi

During the last 35 years the genetic of fungal pathogen population of different crops have been extensively analysed (Bayraktar et al., 2007; Clay, 1995; Dinolfo et al., 2010; Goodwing et al., 1995; Stenglein & Balatti 2006; Stenglein et al., 2010; Mc Dermot & Mc Donald, 1993; Moreno et al., 2008, 2009; Tóth et al., 2004). The genetic variability is frequent among plant pathogen populations and its understanding can aid to diseases management decisions (Brown & Wolfe, 1990; Clay, 1995; Milgroon et al., 1992). The plant pathogenic fungi included a heterogeneous group of organisms occuping positions of great importance in both agriculture and plant communities. The range of pathogens found on different hosts also shows considerable diversity that may be associated with the evolutionary history of their host (Clay, 1995) or ecological criteria like the host's architectural complexity or the extent of the pathogen's natural range (Strong & Levin, 1979). The plant pathogenic fungi rely on the processes of mutation and recombination as the ultimate source of genetically based variation (Burdon & Silk, 1997). Burdon and Silk (1997), suggested the different sources and patterns of diversity in plant pathogenic fungi (mutation, migration, gene flow and recombination). The frequency of mutation is highly variable and dependent of rate mutation and ploidy level of pathogen, the size of pathogen population and the selective advantage conferred by mutant phenotype (Burdon, 1997). For example, the origin of the *Puccinia striiformis* in Australia (Welling & Mc Intosh, 1990) is product of mutation process; also, *Phytophtora infestans* in United States (Goodwing et al., 1995). Another mechanism by which one pathogen can be differentiate one pathotype is gene flow, one example of this case was the introduccion of *Puccinia graminis* f. sp. *tritici* in Australia (Burdon & Silk, 1997). The installation of new areas crops can produce fluctuations in the genetic structure of populations of hosts as well as from pathogens (Mc Dermot & Mc Donald, 1993). The level of genetic diversity in fungi is usually the highest in isolates obtained from the center of origin. The genetic variability is a present condition in wild pathosystem that is incrementing in the agroecosystems. Different views (asexual and sexual) that have many of the fungal phytopathogen, provide variability and may affect the genetic structure of a population (Brown & Wolfe, 1990; Milgroon et al., 1992). Those fungi that have the ability to reproduce sexually can produce genetically more variable populations than those that only make it asexually (Goodwin et al., 1992).

Why is important to study the genetic structure of pathogen fungal population of plants? The population genetic structure has been show to be important in the management of a fungal plant disease. Determination of the genetic variability in organisms such as fungal pathogens should be an ongoing task for successful breeding programs. The loss of resistance in commercial cultivars, produced by the emergence of new unknown pathotypes has been documented (Araya & Cárdenas 1999; Young & Kelly 1997). It is important to know the existence of races for different pathogens and the relationship of each one with the host, in order to detect genes for resistance. Genetic variation among isolates of a fungus has been examined through a variety of techniques (Arenal et al., 2002; Dorrance et al., 1999; Moreno et al., 2008a,b; Moriwaki et al., 2003; Pioli et al., 2003). Methods commonly used to assess variation in fungal plant pathogen population include virulence, vegetative compatibility, biochemical and molecular analysis.

3.1 Current status of *Pyrenophora tritici-repentis*

It is recognized that *P. tritici-repentis* presents great variability in its genome and pathogenicity (de Wolf et al., 1998). Several studies included tools for detection variability

using of morphocultural, pathogenic, bioquemical and molecular variability. 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 & 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 & Brown 1987, Moreno et al., 2008a; Pujol Vieira dos Santos et al., 2002). It has been noted in these studies that isolates of *P. tritici-repentis* vary greatly.

There are numerous works that refer to the population structure of *P. tritici-repentis* in races (Ali & Francl 2002, 2003; Ali et al., 2002; 2010; Andrie et al., 2007; Lepoint et al., 2010; Manning et al., 2002; Strelkov et al., 2002; Lamari et al., 2003; 2005). Some of these papers involve aspects as morpholocultural and virulence variability (Ali et al., 2010). In general in the mostly combined with molecular markers and PCR (Ali & Francl 2002, 2003; Ali et al., 2010; Andrie et al., 2007; Lepoint et al., 2010; Manning et al., 2002; Strelkov et al., 2002; Lamari et al., 2003; 2005).

Several molecular methods have been used to analyze the diversity of plant pathogens at the genome level, such as random amplified polymorphic DNA (RAPD), amplified fragments length polymorphism (AFLP), enterobacterial intergenic consensus (ERIC), repetitive extragenic palindromic (REP), simple sequence repeats (SSRs) and inter simple sequence repeats (ISSR) (Mehta 2001; Mehta et al., 2004; Pujol Vieira dos Santos et al., 2002; Stenglein & Balatti, 2006).

A preliminary study with Brazilian isolates of *P. tritici-repentis* demonstrated high levels of polymorphisms, but no correlation between RAPD, geographic origin and/or pathogenic data (Pujol Vieira dos Santos et al., 2002). The pathogenic and molecular analyses (ISSR) showed intraspecific variability within *P. tritici-repentis* isolates and it was not possible to establish a relationship between this variability and the geographical regions and/ or wheat cultivars from where *P. tritici-repentis* isolates were obtained (Moreno et al., 2008a). Friesen et al., (2005) using AFLP markers determined a high level of variability among isolates of *P. tritici-repentis* and these results shown no genetic grouping of pathotypes or grouping for geographic location. According to Pujol Vieira dos Santos et al., (2002), there are many factors that could affect polymorphism analysis, e. g. the intra-specific variants of a pathogen, the number of samples selected for analysis, genetic flow between populations, environmental adaptation and adaptation to a new host and selective pressure and migration. On the other hand, *P. tritici-repentis* is a homothallic fungus that readily produces the sexual stage on field stubble, giving this fungal population the opportunity for adaptation by sexual recombination. The occurrence of sexual recombination in nature is likely the reason for the high level of genetic variability among isolates of *P. tritici-repentis* (Singh & Hughes, 2006). Some works have studied the homothallic nature of *P. tritici-repentis* using the specific molecular markers for detection of the MAT genes (Lepoint et al., 2010).Under favourable conditions conidiospores can travel 10-200 km (de Wolf et al., 1998). The tan spot fungus is also seed-borne and thus, long distance travel of fungal inoculum can occur by seed transmission (Singh & Hughes, 2006). Hence, we can assume that the occurrence the sexual state and long distance dispersal of inoculum could have contributed to pathogenic and genetic variability independent of geographic origin.

Recently, primers to detect the gene that codified for specific toxins Ptr ToxA, Ptr ToxB and Ptr toxb have been developed and used for many studies, and several works have enunciated that the classification in races of *P. tritici-repentis* based only on phenotypic symptoms is in disagreement with the obtained results of genotypic analysis (Ali et al., 2010; Andrie et al., 2007).

3.2 Isozymes

Isozyme electrophoretic analysis is a powerful biochemical technique used by mycologist and plant pathologist to asses the genetic variation within and among fungal populations, to trace the geographic origins of pathogens, and to determine ploidy levels in fungi (Brammer, 2000; Bonde et al., 1993; Castro & Bach, 1993; Mc Donald & Mc Dermott, 1993; Micales et al., 1998, 1986; Reynolds et al., 1983). Isozyme analysis has proved to be a successful tool in studying the evolution of races in other fungal species (Bocchese et al., 2003; Burdon & Roelfs, 1985; Fry et al., 1991; Koch & Kölher, 1990). In several of these studies, isozyme markers were of particular helpfulness, because no other genetic markers were available or because the only available markers, viz, virulence genes, were not selectively neutral (Welz et al., 1994).

With isozymes, a large numbers of staining systems can be used for the comparison of the numerous genetic loci coding for enzymes from many metabolic pathways (Bonde et al., 1993). This has the advantage of allow us to draw conclusions about the genetic variability existing within and between species under investigation (Bonde et al., 1993). The selection of enzymes to study is an important part of the enzyme analysis. Some strains, such esterases (EST), phosphatases (ACP) and peroxidases (POX), are not substrate specific and detect entire groups of enzymes (Bonde et al., 1993). These enzyme systems have been used by others authors in previous studies (Chen, 1992; Elias & Schneider, 1992; Petrunak & Christ, 1992).

The analysis of isozyme data showed different resolution for the three enzyme systems used among the 155 *P. tritici-repentis* isolates (Moreno et al., 2008a). Poor resolution was observed for the POX enzyme, while EST and ACP enzymes showed strong and acceptable resolution. *P. tritici-repentis* isolates variability was shown by the analysis of the different isozyme patterns. The highest indications of variability were found with the esterases and acid-phosphatase systems. Peroxidase was less useful to distinguish differences between isolates. The variability within a worldwide collection of isolates *P. tritici-repentis* was studied using pathogenicity test (Ali & Francl, 2001; Lamari & Bernier, 1989a, b; Lamari et al., 1998, 1995; Luz da & Hosford, 1980). According to Ali and Francl (2002) *P. tritici-repentis* has a diverse population in South America. In this study, our collection of *P. tritici-repentis* isolates from Argentina showed a considerable isozyme variation for two of the three systems assayed. This fungus is the predominant necrotrophic pathogen of wheat crops in Argentina and an increase in occurrence of this disease is reported since the least decade (Carmona et al., 1999). Isozyme analysis has been recommended as a possible assistance in the systematic and genetic of fungi (Garber & Behara, 1966; Faure-Raynaud et al., 1987). This technique has also been assayed to characterize interespecific and intraspecific polymorphisms of fungi (Anné-Peberdy, 1981; Boshoff, 1996; Bridge et al., 1986). The use of isozymes as markers for estimating the amount of variability within fungal populations is well documented (Mc Donald & Mc Dermott, 1993; Micales et al., 1998). The lack of genetic

variation within fungal pathogens has previously been correlated with various factors. Newton (1987) reported that a specialized obligate parasite should exhibit little variation due to the uniformity of its substrate whereas facultative pathogens, which are more exposed to diverse substrates and environment will be variable. *P. tritici-repentis* has been reported from or observed on several hosts (Ali & Francl, 2002; Hosford, 1971; Krupinsky, 1992). The isozyme assay revealed considerable diversity among the *P. tritici-repentis* isolates assayed than would be expected from a homothallic fungus (Linde et al., 1997). Matsumura (1991) showed variability among isolates of *Bipolaris sorokiniana* from the same wheat cultivar and from different cultivars. Valim-Labres (1997) observed that the host determined the genotype pathogen. In *Uromyces appendiculatus*, *Gliocladium* sp. and *Pythium* sp. populations, isozyme polymorphisms were observed (Chen et al., 1992; McCain & Groth, 1992). In other pathogens as *Colletotrichum acutatum* and *C. fragariae*, the variability observed was low but high in *C. gloesosporioides* (Bonde et al., 1991).

In several studies, this heterogenicity was associated with the parasitic type exerced by the pathogen (Chen et al., 1992; Elias & Schneider, 1992; Petrunak & Chrits, 1992). Those pathogens with a saprophytic stage have a high diversity of enzymes for use on different substrates (Petrunak & Chrits, 1992). Dorrance (1999) observed that the obligate pathogens showed low levels of isozyme polymorphisms. This observation is attributed because these pathogens live on more uniform substrates than facultative ones. *P. tritici-repentis* is a facultative pathogen whose pathogenic stage can be observed on diverse environments and broather hosts range, like different gramineous species. These observations could explain the results observed in this study for *P. tritici-repentis* for EST and PHOSP systems. The same considerations were proposed by Pujol Vieira dos Santos et al., (2004) for *Bipolaris sorokiniana*. Moreno et al (2008a), showed for *P. tritici-repentis* no association among bands and origin of the isolates. This result was observed by others authors for other pathogens (Chen et al., 1992; Hellmann & Christ, 1991; Pujol Vieira dos Santos et al., 2004). Hellmann and Christ (1991) observed that *Ustilago hordei* isolates from USA and Etiopia, showed the same isozyme pattern. By other hand, Chen et al., (1992) observed groups of *Pythium graminicola* isolates originated from the same localities, but also groups of *P. arrhenomanes* isolates originated from different localities in USA. Bocchese et al., (2003) did not observed relation among isozyme patterns and origin of *Pyrenophora avenae* isolates. Moreno et al., (2008a) suggested further studies of esterases for *P. tritici-repentis*, because within this isozymes, the carboxylesterases, have relation with resistance mechanisms to insecticides, cupper fungicides, plaguicides and fosforate compounds (Bisset, 2002). Furthermore, it would be of interest to study this system in detail due the high polymorphisms observed for argentinian *P. tritici-repentis* isolates. Moreover, Nicholson et al., (1972) showed that microorganisms as *Xanthomonas axonopodis* pv. *manihotis*; *Botrytis cinerea* and *Venturia inaequalis* presented estearase activity near penetration site. Pascholati et al., (1992) observed correlation between presence of estearase and conidia of *Erysiphe graminis*. Bocchese et al., (2003) observed correlation between EST isozyme patterns and virulence of *Pyrenophora chaetomioides*, causal agent of foliar disease on oat. In Argentina, only a few reports are available about electromorphic types of *P. tritici-repentis* based in isozyme analysis (Moreno et al., 2008a).

3.3 Molecular markers

Since 1990, the molecular biology through the use of the PCR technique facilitated the availability of different tools for identification and characterization with high precision

different microorganisms. The PCR technique was developed by Mullis in 1983 (Mullis, 1990). The DNA fingerprinting consists in the display of a set of DNA fragments from a specific DNA sample (Vos et al 1995). It is used to visualized and identify DNA polymorphisms between individuals. This technique is used to determining the identity of a specific, to identify molecular markers linked to phenotypic traits and/or genetic loci to generate a linkage map. It is known that the PCR, use either specific of arbitrary primers to develop the *in vitro* amplification of template DNA. The different amplification patterns are a function of the primer sequence and the nature of the template DNA. The molecular markers have been used to evaluate genetic diversity in and between species of fungal plant pathogens (Kalkar et al., 2006; Moreno et al., 2009; Saharan & Naef 2008; Stenglein & Balatti, 2006; Stenglein et al., 2010; Yli-Mattila et al., 2004). DNA fingerprint allows visualize and indentify DNA polymorphisms between fungi.

Strelkov and Lamari (2003), published a review where indicated the new advances in the study of wheat- *P. tritici-repentis* interaction from 1999 to 2003. In this review they suggested the existence of eight races of *P. tritici-repentis* determined on four lines of wheat (Glenlea, 6B662, 6B365 and Salomouni). They considered that the molecular basis of virulence has been explained, with the identification of three toxins. The reaction of the host to pathogen is controlled by single dominant and independently inherent genes, suggesting a one-to-one relationship between wheat- *P. tritici-repentis*. In the same work they highlighted the importance that the genes codified for these toxins (*Tox* A, *Tox* B, *tox* b) have been cloned and provided the basis for further works. Also, Singh et al., (2010) published a review with the last advances in the characterization of wheat- *P. tritici-repentis* interactions. None of three reviews proposed a point about the genetic intraspecific variability of *P. tritici-repentis* utilizing the use of molecular markers. However, at present exists different studies where the aim consists in the characterization of genetic variability of *Pyrenophora tritici-repentis* population in different localities, areas and countries. (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 & Hughes, 2006).

During the last ten years, several isolates obtained from the both same and different area of *P. tritici-repentis,* have been study with different molecular markers. Within them Amplified Fragment Length Polymorphism (AFLP) (Friesen et al., 2005; Leisová et al., 2008), Enterobacterial Repetitive Intergenic Consensus (ERIC) and Repetitive Extragenic Palindromic (REP)- PCR technique (Mehta et al., 2004), Internal Transcribed Spacer Regions (ITS) (Lepoint et al., 2010), Mating Type locus (MAT) (Lepoint et al., 2010), Inter Simple Sequence Repeat (ISSR) (Moreno et al., 2009) and Random Amplified Polymorphisms DNA (RAPD) (Pujol Vieira dos Santos et al., 2002; Singh & Hughes, 2006), Restriction Fragment Length Polymorphism (RFLP), Toxin production genes (*Tox* A, *Tox* B and *tox* b genes) (Ali et al., 2010; Andrie et al., 2007; Lepoint et al., 2010).

3.3.1 Restriction Fragment Length Polymorphism (RFLP)

During the past few years also work with the procedure known as Restriction Fragment Length Polymorphism (RFLP) is used for characterization of plant pathogen fungi. Isolates of different species of have been characterized used this methodology (Arabi & Jawhar, 2007; Wu et al., 2003; Zein et al., 2010). This procedure was developed by Botstein et al.,

(1980). The strategy of this technique is the hybridization: consist in the detection of fragment of genomic DNA cut with restriction endonucleases, separated based on their size by electrophoresis and identified by Southern Hybridization with specific labelled probes. This technique offers an alternative means by which genetic diversity can be measure in pathogens (Kohli et al., 1992; Mc Donald & Mc Dermott, 1993; Milgroom et al., 1992; Mueller et al., 1996; Peever & Milgroom, 1994; Zhong & Steffenson, 2001). Wu et al., (2002), showed that every *P. teres* isolates obtained from geographically diverse collection, exhibited a unique RFLP pattern. However, the same isolates showed a broader spectrum and a higher level of virulence on the host differentials (Wu et al., 2003). Recently, Zein et al., (2010), analyzed isolates of *P. graminea*, the causal agent of leaf stripe disease obtained from Syria, using ITS-RFLP. They observed the fingerprints generated from the six restriction digestions of the DNA ITS region showed high levels of intraspecific variation within the *P. graminea* population. Zein et al., (2010), used IRAP (inter-retrotransposon amplified polymorphism) and ITS-RFLP, each marker system could discriminate between all of the isolates in detecting polymorphism. Similar studies with a global collection of *P. tritici-repentis* could be developed to provide information for future selection of isolates to develop durable wheat tan spot resistance.

3.3.2 Amplified Fragment Length Polymorphisms (AFLP)

AFLP, have been used in different works for characterize the population of different *Pyrenophora* species (Friesen et al., 2005; Leisová et al., 2005, 2008; Serenius et al., 2007). This procedure is based on the selective amplification of genomic restriction fragments. The AFLP techniques, generated fingerprint without previous knowledge of the sequence using a limited set of generic primers (Vos et al., 1995). It is a robust and reliable technique, based on the high reproducibility, and on the homology between bands of the same weight. In the case of causal agent of tan spot, only two researches worked with this technique (Bentata et al., 2011; Friesen et al., 2005; Leisová et al., 2008). Friesen et al., (2005), presented a study of genetic diversity of global collection of *P. tritici-repentis*. They analyzed different isolates (97) obtained from Canada, Czech Republic, United States of America (Arkansas, Kansas and Oklahoma) and South America (Argentina, Brazil and Uruguay). They observed that the level of genetic diversity detected by AFLP was "*reasonably*" high, were not able to group isolates of fungal races or geographic location. They suggested that these results were obtained because the fungi have preferentially out crossing in nature and the fungus possibly can be travel in seed movement between continents. Similar study was developed by Leisová et al., (2008). They analyzed fingerprinting resulted of AFLP analyses of 100 *P. tritici-repentis* isolates obtained from Canada, Czech Republic, Russia, Slovak Republic, United States of America and Argentina (South America). They observed that the most variability occurred within local population, and among population this variability is lesser. They attributed these results to sexual reproduction and that *P. tritici-repentis* is a seed pathogen and it can travel long distances. The studies suggested that the AFLP technique is appropriate to detect the intraspecific variability for *P. tritici-repentis* population, and that the genetic variability is most than race structure or/and geographic origin. This type of molecular markers did not reveal a positive correlation among race and geographic origin with identified haplotypes. Similar number of isolates obtained from of different wheat area around the world could be compared with this technique.

3.3.3 Random Amplified Polymorphisms DNA (RAPD)

RAPD-PCR is performed at low annealing temperatures in order to allow primers to anneal to not fully homologous loci. The RAPD technique is quick, effective and produces reliable markers which have been used for the identification of various fungal pathogens. RAPD analyzes the entire genome the fungus, this technique has been extensively used for characterization of plant pathogen fungi (Assigbetse et al., 1994; Bayraktar et al., 2007; Guthrie et al., 1992; Tóth et al., 2004; Pujol Vieira dos Santos et al., 2002; Di Zinno et al., 1998; Singh & Hughes, 2006). In general, it is useful in detecting inter and intra-specific variation. Within the genera *Pyrenophora* the use of RAPD to detect intraspecific variability was used by several researches (Campbell & Crous, 2002; Mehta, 2001; Peever & Milgroom, 1994; Pujol Vieira dos Santos et al., 2002; Singh & Hughes, 2006). Mehta (2001), observed intraspecific variability among isolates of *Dreschlera avenae* obtained from both black spot and leaf spot symptoms. Mehta (2001) suggested that the difference observed between the isolates originating from two types of symptoms is due to intraspecific variants of *D. avenae*. Pujol Vieira dos Santos et al., (2002), analyzed the intraspecific diversity of *P. tritici-repentis* isolates obtained from seeds in Brazil. The RAPD analyses showed intraspecific polymorphisms within the isolates, but it was not possible to establish a relationship between these polymorphisms and the geographical regions where the host seeds were collected. Guthrie et al., (1992) with isolates of *Colletotrichum graminicola* and Assigbetse et al., (1994) with isolates of *Fusarium oxysporum* f. sp. *vasinfectum* were able to detect a relationship between intraspecific polymorphisms and geographic location. Sing & Hughes (2006), published the results obtained from RAPD analyzes of *P. tritici-repentis* isolates. They observed, the level of polymorphisms detected of analysis of molecular variation (AMOVA) was of 96.8%among isolates, but none differentiated isolates of *P. tritici-repentis* on the basis of race classification or geographical origin. Several studies (Crous et al., 1995; Di Zinno et al., 1998; Pujol Vieira dos Santos et al., 2002; Mehta, 2001; Singh & Hughes, 2006) and highlighted the high level of genetic variability among isolates of *P. tritici-repentis* and that this genetic variability was independent of race classification or geographical origin.

3.3.4 Enterobacterial Repetitive Intergenic Consensus (ERIC) and Repetitive extragenic palindromic (rep-PCR)

The ERIC and REP-PCR technique is a rapid and highly reproducible method that involves PCR amplification using primers that correspond to ERIC and REP elements (Versalovic et al., 1991). Identifying families of repetitive DNA sequences has been show also to be an useful and reliable strategy to determine the genetic relationships within groups of microorganisms. Although rep-PCR are used to identify highly conserved, repeated nucleotide sequences in bacterial DNA (Hulton et al., 1991), Gillings and Holley (1997) reported that rep-PCR fingerprinting can also amplify other regions and even non-bacterial sequences of the bacterial genome . Despite this limitation, rep-PCR fingerprinting has been used to analyze the DNA of various fungal species (Edel et al., 1995; Mehta et al 2004; Reynaldi et al., 2003). Mehta et al., (2004) working with 40 isolates of *P. tritici-repentis*, and showed that the ERIC-PCR and rep-POCR patterns for almost all the isolates were homogeneous, suggesting that the genetic variability of *P. tritici-repentis* population in this area in Brazil (State of Paraná) was small. However, Mehta et al., (2002), using ERIC and rep-PCR to analyze de genetic variability among isolates of *Stemphyllum solani*, found clear

differences between the cotton and tomato isolates as well between the tomato isolates from different geographic regions. Redondo et al., (2009) applied these techniques to discriminate *P. glabrum*, *P. adametzioides* and *P. thomii* from other *Penicillium* species. In Argentina, future studies should be order to validate the ability of these techniques to distinguish between races of *P. tritici-repentis*.

3.3.5 Inter Simple Sequence Repeat (ISSR)

ISSR consists of the amplification of DNA sequences between SSR by means of anchored or non-anchored SSR homologous primers (Zietkiewicz et al., 1994). SSR are tandem repeat motifs composed of one to six nucleotides, which are ubiquitous, abundant and highly polymorphic in most eukaryotic genomes (Tautz & Renz 1984). This technique involves the use of one primer complementary to a target microsatellite region in PCR and allows DNA amplification between microsatellite regions. ISSR does not require a previous knowledge of the sequence and generates specific and reproducible patterns due to the high stringent conditions of annealing (Bornet & Branchard, 2001). Several authors has been using this technique to determine the genetic variability in plant pathogenic fungi (Camacho & Liston, 2001; Dinolfo et al., 2010; Moreno et al., 2009; Stenglein & Balatti, 2006; Statkevi´ciute et al., 2010). Within Ascomycota fungi, Stenglein and Balatti (2006), observed a high genetic variability among isolates of *Phaeoisaripsis griseola*. Regarding the genera *Pyrenophora*, Statkevi´ciute et al., (2010) used ISSR to determine the genetic diversity of *P. teres* obtained from Lithuania. They observed that all isolates had a unique ISSR haplotype. However, Moreno et al., (2008b) observed several ISSR haplotypes for *P. tritici-repentis* isolates obtained from Argentina, using five of 28 ISSR primers tested. Dinolfo et al., (2010), detected similar results for other necrotrophic fungus, *Fusarium poae* and Misrha et al., (2004) for *F. graminearum*. In all these works, the conclusion was the high intraspecific variability, but they did not reveal a clear relationship between variability and the host/geographic origin. Moreno et al., (2008b), suggested that the genetic variability at the DNA level among isolates of *P. tritici-repentis* based in ISSR markers was unrelated with pathogenic variability. In agreement with Sicard et al., (1997) and Stenglein and Balatti (2006), we considered that isolates with the same pathogenicity patterns are not necessarily closely related based on DNA analysis.

3.3.6 Internal Transcribed Spacer regions (ITS)

Some genera of fungi are intensively studied and characterized with the use of the ITS region. DNA sequence data from ITS region demonstrated that is the indispensable information source in the taxonomy of fungi, in the diversity of fungi under different scales of time and space (Horton & Bruns, 2001; Hsiang & Wu, 2000; Redecker, 2000; White et al., 1990). It has been used to distinguish between closely related fungal isolates. The ITS region has been used wide because it is a noncoding region and its size and sequence are less conserved, it is used to examinate related population of fungi (Pritsch et al., 1997). Green et al., (2004), analyzed different set of primers for ITS region and 18 S rDNA to design specific primers to Pyrenomycetes and to determine the utility of ITS region for genotyping Pyrenomycetes isolates. Phylogenetic studies of genera *Pyrenophora* has been developed by Zhang and Berbee (2001). Arabi and Jawhar (2007) observed high genetic diversity in Syrian population of *P. graminea* with ITS region. Zein et al., (2010) observed fingerprints generated

from the five restriction digestions of the nrDNA ITS region denoted high levels of intraspecific variation within the *P. graminea* population. Bakri et al., (2011) observed high levels of intraspecific variation within the *P. graminea* population. Arabi and Jawhar (2007) and Bakri et al., (2011) suggested the use of other markers to better clarify genetic diversity in Syrian population *P. graminea*. In general, the used of ITS region to study *P. tritici-repentis* has been used in combination with others region as nuclear gene coding region glyceraldehido-3-phosphate dehydrogenase (*gpd*) and mating type locus (*MAT*) (Lepoint et al., 2010). They suggested that with ITS region and *gpd* gene did not detected genetic variability among 20 isolates of *P.tritici-repentis* obtained from different countries. These sequences suggested that *P. bromi* is one of the *P. tritici-repentis`s* closest relatives. In the general use of ITS region and others region of rDNA are use for phylogenetic studies genera level. This technique has been used in the last years for detection and follow sequenciation of specific fragment of DNA fungi and revealed by denaturing gradient gel electrophoresis from environmental samples (Anderson et al., 2003; Green et al., 2004). Since the ITS region is highly conserved intraspecifically but variable between different species it is often used in taxonomy (Bruns et al., 1991; Hillis & Dixon, 1991).

3.3.7 Mating type locus (*MAT*)

The study of genetic diversity through of the mating type genes (*MAT*) allowed to clarify the population genetic structure of some plant pathogens fungi and provided the opportunity to evidence the sexuality reproduction in several fungi (Bennett et al., 2003; Lepoint et al., 2005, 2010; Rau et al., 2005; Serenius et al., 2005).

The mating-type genes of *P. teres* have been studied by different researchs (Rau et al., 2005; Serenius et al., 2005). The *P. teres* isolates obtained from Italy showed two *MAT* genes (1:1), suggesting that the sexual reproduction is the major influence in the population structure. On other hand, Serenius et al., (2005) observed a high variability between regions suggesting that the sexual reproduction occurs but its influence on the population structure is relative, and may be based in environmental differences. The taxonomic status of formae speciales of *P. teres* was proposed for reconsideration by Rau et al., (2005). A similar study of Rau et al., (2005) and Serenius et al., (2005) was developed by Lepoint et al., (2010) with isolates of *P. tritici-repentis* obtained from naturally infected leaves since 1980s in wheat-growing areas worldwide. They showed that all isolates amplified to *MAT* gene and they highlighted some points: a) the organization of *MAT* gene the *MAT1-1* gene is followed by the *MAT1-2* gene, with both ORFs transcribed left to right; b) some strains presented one fragment as insertion between primer PtrPLP5 and PtrPLP6 (Lepoint et al., 2010) in a variable region; c) some strains of *P. tritici-repentis* revealed a nonspecific profile with the primer par PtMat_fw (Rau et al., 2005) and PtrPLP2 (Lepoint et al., 2010). They concluded that *P. tritici-repentis* has a simple *MAT* locus organization containing both idiomorphs tandemly arranged in a single individual. This result corroborated the homothallic condition of *P. tritici-repentis*. They could differentiate two groups, one group more homogeneus including isolates obtained from different areas worldwide; the other one including isolates of *P. tritici-repentis* classified as race 4 and in the other groups these isolates are lack. Also in the second group they observed the presence of others isolates of *P. tritici-repentis* classified as race 1 and 2 (Lepoint et al., 2010). They suggested that the clear-cut is due to the presence of isolates of race 4 in only one group. However, the number of isolates classified as race 4 is low respect

the others races. Lepoint et al., (2010) suggested the new distinction between the strains of *P. tritici-repentis* and they proposed the revision of the intra-infraspecific taxonomic status of the pathogen. They suggested that the MAT is the first gene follow the *HTS* gene that has been able to distinct the different lineages within *P. tritici-repentis*.

3.3.8 Toxin production genes (*Tox* A, *Tox* B and *tox* b)

The technique wider used to the characterization of *P. tritici-repentis* population has been based in the interaction wheat-*P. tritici-repentis* (Ali & Francl, 2001$_{a,b}$, 2002$_b$; Lamari & Bernier, 1989$_{a,b}$; Lamari & Gilbert, 1998; Lamari et al., 1995, 1998, 2003, 2005) add the phenotypic characterization in races. Due the subjective of this technique add the availability of the sequences of the *Tox* genes (Ballance et al., 1996; Ciuffetti et al., 1997; Martinez et al., 2001; 2004). Different researches has been taken the sequences for construction of different specific primers to detect the presence of Tox genes in the different strains of *P. tritici-repentis* (Ali et al., 2010; Andrie et al., 2007; Martinez et al., 2001; 2004; Moreno et al unpublished). The *ToxA* gene codified to the production of Ptr ToxA, this toxin produces necrotic symptoms in Glenlea and Katepwa and must be present in the strains classified as races 1, 2, 7 and 8. The Ptr Tox B is produced by races 5, 6, 7 and 8; and races 1, 3, 6 and 8 produce Ptr Tox C. The race 4 is nonpathogenic and do not produces any symptoms. The races 5, 6, 7 and 8 produce chlorosis in Katepwa and 6B662. In this case of Ptr Tox B is encoded by the multiple copy of *ToxB* gene (Martinez et al., 2001, 2004; Strelkov et al., 2006). It is know that the races 3 and 4 have present in their genomes singles copies of distinct *ToxB* homolog (Martinez et al., 2004; Strelkov et al., 2006). Therefore found *P. tritici-repentis* strains carrying the *ToxB* gene but without production of Ptr ToxB were found (Andrie et al., 2007; Lamari et al., 1995; Martinez et al., 2004; Strelkov et al., 2006). In this way and considering that the virulence patterns of the various *P. tritici-repentis* isolates are based in that each compatible interaction host-pathogen that it is mediated by HST (Strelkov & Lamari, 2003); Andrie et al., (2007) suggested that the use of PCR multiplex is appropriated to corroborate the phenotypic identification of *P. tritici-repentis* races. In their manuscript their suggested suggests the erroneous identification for two strains of *P. tritici-repentis* (SO3 and PT82), the phenotypic assignation for this strains corresponded with race 2 and 8 respectively. Specifically they observed that SO3 lacks the *Tox A* gene and Ptr Tox A production and PT82 lacks *ToxB* gene and Ptr Tox B production. This point is fundamental to their recommendation "*results suggested that genotypic confirmation shoul accompany, where possible, phenotypic characterization when designating a P. tritici-repentis isolate to race*" (Andrie et al., 2007). According the previous work of Andrie et al., (2007), other publications refers studies of *P. tritici-repentis* population structure in races (Ali et al., 2010; Lepoint et al., 2010; Moreno et al., (unpublished data 2011)). Ali et al., (2010) tested 42 isolates obtained from Arkansas (United State of America) to the presence or absence of the *ToxA* and *ToxB* gene. Their results are agreement with the results of Andrie et al., (2007). Some isolates ToxA gene deficient caused necrosis on Glenlea and others that did not cause necrosis. Then, they suggested that isolates of *P. tritici-repentis* deficient in *ToxA* and T*oxB* gene and that induce necrosis and/or chlorosis may produce a novel toxin(s) on wheat (Ali et al., 2010). In the same year, Lepoint et al., (2010) tested the presence of *HTS* gene and others markers as ITS, *gpd* gene and *MAT* gene to determine the genetic diversity of an extensive collection of *P. tritici-repentis* isolates. In basis of the results obtained they observed discrepancies between both methods, and they highlighted the potential existence of novel(s) toxins and new races.

Similar results were observed by Moreno and colleagues (unpublished) for isolates of *P. tritici-repentis* obtained from Argentina.

4. Discussion and future prospect

Wheat is one of the most important crops in the world. Global wheat supplies for 2011/12 are projected 11.4 million tons higher with higher beginning stocks and a sharp increase in production (font: WASDE – 497 United States Department of Agriculture ISSN: 1554-9089). The future of this production and its participation of the international markets depend on the evolution of proposal of different countries. It is clear that, the start in the field and the exportation of the crop depend on the introduction of new technological develops. Among these developments are the new cultivar composition, management of crops and management of future areas for yield expanding (Ekboir & Morris 2001). The fungi pathogens are become by the combination of these factors (Klein 2001). The management of these diseases to ask the knowledge specific and the increased ability to identify the fungus and the techniques for reduce to minimum the losses of crops (Kohli, 1995). Tan spot disease began to noticeably affect wheat crops region around the world (Annone, 1985; Duveiller et al., 1998; Lamari et al., 2003). It is well known the existence of a fourth HST, additional race that has also been suggested (Andrie et al., 2007; Ali et al 2010; Moreno et al., (unpublished data)). In some studies it has been observed discrepancies between both pathogenic and genetic methods to determine the races (Andrie et al., 2007; Ali et al., 2010; Lepoint et al., 2010; Moreno et al., (unpublished data)). Moreover, several works determined a high level of variability among isolates of *P. tritici-repentis* and these results shown no genetic grouping of pathotypes or grouping for geographic location. These results suggested that the population of *P. tritici-repentis* is complex in reaction types and this characteristic should be considered. The different works cited above have corroborated the intraspecific variability of *P. tritici-repentis* using different techniques. This intrasepecifc variability is not high enough to consider the revision of taxonomic status of *P. tritici-repentis*. However, Lepoint et al., (2010) based on their results with *MAT* genes suggested that could be also be several distinct taxa. Population diversity and genetic structure are two important factors in the management of plant disease. The variability in the population of plant pathogen fungi can be very highly, if we know the degree of variability we could foresee the potential occurrence of new races, speciation process and new isolate fungicide resistance. The pathogenic and genetic analysis of *P. tritici-repentis* showed intraspecific variability. However, in any case was possible to establish a relationship between this variability and the geographical origin or physiological race. In some analyses, it was shown that *P. tritici-repentis* isolates from the same region appeared in the same group. Due the last studies, and the discrepancies showed between methods to determine the population race structure is that we suggested an exhaustive genetic study of a worldwide collection of *P. tritici-repentis* isolates, with similar number of isolates and races for countries in order to obtain a clear genetic mapping of *P. tritici-repentis* population around the world.

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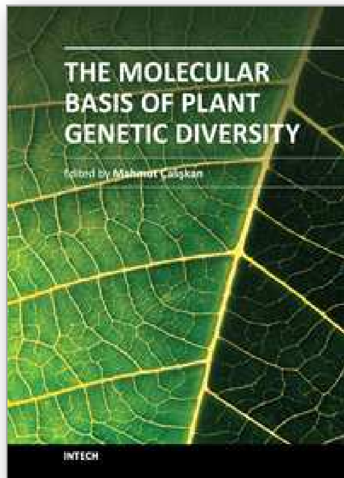

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The Molecular Basis of Plant Genetic Diversity presents chapters revealing the magnitude of genetic variations existing in plant populations. Natural populations contain a considerable genetic variability which provides a genomic flexibility that can be used as a raw material for adaptation to changing environmental conditions. The analysis of genetic diversity provides information about allelic variation at a given locus. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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Phone: +86-21-62489820
Fax: +86-21-62489821

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