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Patterns of virulence diversity in *Puccinia recondita* on wheat in Morocco in 2005 and 2006

FARIDA BOUFTASS[1], BRAHIM EZZAHIRI[2] and AZIZ ZIOUTI[1]

[1] *Laboratoire de Biochimie & Biologie Moléculaire, Département de Biologie, Faculté des Sciences Ain Chock, Université Hassan II, BP 5366-Maârif, Casablanca, Morocco*
[2]*Département de Phytopathologie, Institut Agronomique et Vétérinaire Hassan II, BP 6202- Madinat al irfane, Rabat, Morocco*

Summary. A total of 105 isolates of *Puccinia recondita* from durum wheat and common wheat were collected from the four main agro-ecological areas of Morocco, Abda-Doukkala, Chaouia-Tadla, Gharb-Saïss and Tangérois. The isolates were tested for virulence phenotypes on seedling plants of 21 near-isogenic lines of Thatcher wheat. Eighty-nine virulence phenotypes were identified and the resistance genes *Lr2a*, *Lr2b*, *Lr2c*, *Lr3bg*, *Lr3ka*, *Lr9*, *Lr21*, and *Lr24* were found to confer a good resistance on isolates of all four collections. In the set of differentials used in this study, no significant difference was found between virulence frequencies of isolates from durum and from common wheat. Principal coordinates analysis and the Kosman distance between virulence phenotypes showed that the collections from Gharb-Saïss and Tangérois were closely related to each other, while Abda-Doukkala was closely related to Chaouia-Tadla.

Key words: leaf rust, wheat, virulence phenotypes.

Introduction

Rust diseases caused by *Puccinia* spp. have a significant impact on cereal production worldwide because these species rapidly evolve new virulent pathotypes on previously resistant cultivars (Keiper *et al*., 2006). Rust fungi are highly successful obligate parasites of plants and important pathogens of wheat (Anikster and Wahl, 1979), and leaf rust is a major disease of common wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L. var. *durum*) that occurs regularly wherever wheat is grown (Sambroski, 1985).

Leaf rust spreads as clonally produced dikaryotic urediniospores, which can be wind-blown for thousands of kilometres from the initial infection site. Epidemics of wheat rust can occur on a continental scale because of the widespread dispersal of the urediniospores (Roelfs, 1989). The commonly found alternate host of leaf rust in the temperate zones is *Thalictrum speciosissimum* L. (in the Ranunculaceae). But *Anchusa italica* Retz. (in the Boraginaceae) was reported as a functional alternate host for leaf rust of wheat in Morocco (D'Oliveira and Sambroski, 1966; Ezzahiri *et al*., 1992; Ezzahiri *et al*., 1994).

Based on differences in the pycnial-aecial and the uredial-telial host range, Anikster *et al*. (1997) distinguished two groups (I and II) of *Puccinia recondita* collections. Collections of each of these groups were interfertile only with collections of the same group but not with collections of the other group. This shows that these two groups are genetically isolated from each other. Group I contains collections that have *Thalictrum speciosissimum* as the principal pycnial-aecial host. Group II has collections that have one or more species in the Boraginaceae as the pycnial-

Corresponding author: F. Bouftass
Fax: + 212 522230674
E-mail: farida_bouftass@yahoo.fr

aecial host. In group II, type A which includes a Moroccan collection of *P. recondita* (Ezzahiri *et al*., 1994; Bouftass *et al*., 2009), was compatible with *Triticum turgidum* as well as with *Triticum aestivum*. The other collections from wild wheats within group II did not include cultivated wheats and were classified as types B, C and D.

Planting wheat cultivars with genetic resistance to leaf rust is the most practical method of controlling this disease. Effective leaf rust resistance in wheat cultivars is dependent on the virulence of the regional populations of the leaf rust (Kolmer *et al*., 2003).

Dyck and Sambroski (1974) developed a series of near-isogenic lines of the wheat cultivar Thatcher that differed by single leaf rust resistance genes, and their use has made it possible to distinguish leaf rust isolates that differ by a single virulence gene (Sambroski and Dyck, 1976). Virulent races of the leaf rust can easily overcome the resistance that has been bred into commercial hexaploid (AABBDD) common wheat cultivars; on average, this occur only 3 years after their release (Singh *et al*., 2004). In the case of tetraploid (AABB) durum wheat, however, resistance to leaf rust has proved to be longer lasting (Ordoñez and Kolmer, 2007).

The traditional means to assess genetic variations in the wheat leaf rust fungus and in cereal rust fungi generally has been by determining their virulence to host differential lines or cultivars (Felsenstein *et al.,* 1991). The objectives of this study were to characterize the virulence of leaf rust collections in Morocco in 2005–2006, and to evaluate the phenotypic diversity within and between Moroccan geographic locations, using the agro-ecological areas Abda-Doukkala, Chaouia-Tadla, Gharb-Saïss, and Tangérois as a case study.

Materials and methods

Wheat leaf rust isolates were collected from Moroccan commercial wheat fields in 2005 and 2006, from four agro-ecological areas (Abda-Doukkala, Chaouia-Tadla, Gharb-Saïss and Tangérois). In Abda-Doukkala the climate is semi-arid; the soils near the Atlantic coast are mostly rendzinas associated with lithosols that have a caliche layer, while the soils further inland are mostly isohumic and vertisols. Cereals represent 80% of the cropping system, and the wheat crop is grown with or without irrigation under marginal production conditions. Chaouia-Tadla is an irrigated plain at the foot of the southern part of the middle Atlas mountains. The climate in this area is also semi-arid, and soils are mostly rendzinas associated with brown soils. The cropping system is based on cereals, particularly barley, but also wheat and maize (80%). In Gharb-Saïss the climate is mild, soils are mainly gleysols and vertisols, and cereals represent 32% of the cropping system. The Tangérois area is hilly and has high rainfall, traditional cultivars predominate, and cereals represent only 7% of the cropping system. In all, 105 isolates were collected (55 isolates from common wheat and 50 isolates from durum wheat), divided among these areas as follows: 21 from Chaouia-Tadla, 44 from Abda-Doukkala, 29 from Gharb-Saïss and 11 from Tangérois. Each collection was made up of isolates from both durum and common wheat and analyzed without regard to the crop from which they came.

The same roads were taken on each year of the field survey. When wheat fields were grouped together in an area, sampling stops were made every 10 km; when fields were scattered, stops were made at least 10 km apart. Surveys were conducted in late March and early April in Abda-Doukkala and Chaouia-Tadla, and in late April and early May in Gharb-Saïss and Tangérois.

From each sample urediniospores were collected with a cyclone spore collector into a size-00 gelatine capsule and suspended in light mineral oil. The spore suspension was atomized onto 7-day-old seedlings of the susceptible common wheat cultivar 'Fertas' in order to increase the inoculum. After allowing the suspension to dry (30 min), the plants were placed in a dark dew chamber overnight and then transferred to a climate chamber (12-hr day at 22°C) in individual booths. After 7 to 10 days, urediniospores were collected into size-00 gelatine capsules, mixed with mineral oil and then applied to a set of differential hosts of near-isogenic lines with single resistance genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr3bg, Lr3ka, Lr9, Lr10, Lr14a, Lr14b, Lr15, Lr17, Lr18, Lr20, Lr21, Lr22, Lr23, Lr24, Lr25,* and *Lr26*. The inoculation technique was the same as that used for 'Fertas'. Infection types (ITs) were recorded after 12 days following the method of Stakman *et al*. (1962). The program HaGis Spreadsheet V.3.1 for automatic Habgood-Gilmour calculation (Herrmann *et al*., 1999) was used to assign races, the 21 differentials were arranged in 7 sets of three, and to each isolate a 7-digit code was assigned. The program HaGis also made it possible to calculate the virulence frequencies of each collection, and the mean virulence complexities (average number of virulence) of each collection. A 0–100% scale was used to measure the virulence frequency, in which: 0–20%, low virulence frequency, 21–50%, moderate virulence frequency, and 51–100 %, high virulence frequency.

Principal component analysis was carried out using GenALEx6 (Peakall and Smouse, 2006), and second dimension scatterplots were generated. The KOIND program (Schachtel and Kosman, 2002) was used to calculate the

diversity of the virulence phenotypes and the distance between collections. Diversity within collections was measured using the normalized Shannon index of diversity and the Kosman index of diversity; both indices range from 0 to 1. The normalized Shannon index of diversity (Marasas *et al*., 2004) was calculated as $Sh(A) = -\sum p_i \ln(p_i)/\ln(n)$, where p_i= frequency of the *i*th virulence phenotype and n = total number of isolates in population *A*. The Kosman index (Kosman, 1996) was calculated as $KW(A) = ASS_{max}(A,A)/n\lambda$, where ASS_{max} is the maximum value of the sum of the distances between n matched pairs of isolates within population *A*, n corresponds to the total number of isolates in *A*, and λ is the number of differentials tested.

The distance between collections was calculated using the Kosman distance index as $KB(A,B) = ASS_{min}(A,B)/n\lambda$, where ASS_{min} is the minimum value of the sum of distances between n matched pairs of an equal number of isolates from populations *A* and *B*, and λ is the number of differentials tested. Calculations for all indexes were made using the bootstrap procedure of the KOIND program that generated random samples of 100 isolates based on the original data.

The paired t-test was used to compare virulence frequencies of isolates of *P. recondita* from common wheat and durum wheat at the 5% probability level, and an ANOVA test was carried out to compare virulence frequency distributions of the four collections at the 5% probability level.

Results

Characterization of virulence phenotypes

In all, 89 virulence phenotypes were characterized out of 105 isolates (Table 1). Five virulence phenotypes were isolated from more than one collection. The mean virulence complexities of Abda-Doukkala, Chaouia-Tadla, Gharb-Saïss and Tangérois were 6, 6, 3.28 and 3.71 respectively.

Virulence frequencies

The paired t-test at 5% probability level for the virulence frequency distributions of the Moroccan leaf rust isolates from durum and common wheat (Table 2) tested in this study showed no significant difference between the two distributions. When an ANOVA test at 5% probability level was carried out with virulence frequency distributions of Abda-Doukkala, Chaouia-Tadla, Gharb-Saïss and Tangérois, significant differences were detected between collections. The virulence frequencies to genes *Lr1*, *Lr14b*, and *Lr15* were low in the isolates from Tangérois and Gharb-Saïss, but moderate in the isolates from Abda-Doukkala and Chaouia-Tadla. Isolates from Chaouia-Tadla had higher virulence frequencies to genes *Lr3*, *Lr20*, and *Lr23* than isolates from Abda-Doukkala, Gharb-Saïss and Tangérois, while isolates from Chaouia-Tadla and Abda-Doukkala showed higher virulence frequencies to genes *Lr17* and *Lr18* than isolates from Gharb-Saïss and Tangérois. Isolates from the four collections exhibited moderate virulence frequencies to genes *Lr14a*, *Lr22*, and *Lr25*, but the genes most resistant to the Moroccan collections were *Lr2a*, *Lr2b*, *Lr2c*, *Lr3bg*, *Lr3ka*, *Lr9*, *Lr21* and *Lr24*.

Diversity within and between collections

Using the standard Shannon index (Table 3), collections from Abda-Doukkala and Gharb-Saïss had the greatest phenotypic diversity, while the collection from Tangérois was the least diverse. The Kosman diversity index showed that collections from Abda-Doukkala and Chaouia-Tadla were the most diverse, whereas the collection from Tangérois was the least diverse.

With the Kosman distance index (Table 4), the collections from Gharb-Saïss and Tangérois differed the least, and those from Abda-Doukkala and Chaouia-Tadla were the next least diverse.

Principal coordinate analysis

When principal coordinate analysis (PCoA) was performed (Figure 1), the first three eigenvectors explained 71.50% (1st 45.67%, 2nd 13.53%, and 3rd 12.31%) of variation, and most isolates from Gharb-Saïss and Tangérois were grouped together.

Discussion

The virulence frequencies of isolates of *P. recondita* from durum and common wheat did not differ significantly from the set of differentials used in this study. These findings are inconsistent with a study of Moroccan *P. recondita* isolates by Ezzahiri *et al*. (1994), who however used a different differential set from that used in this study (*Lr1*, *Lr2a*, *Lr2c*, *Lr3Ka*, *Lr9*, *Lr10*, *Lr11*, *Lr16* and *Lr17*). These authors found that durum wheat isolates were less virulent than common wheat isolates. A study of a worldwide collection of *P. triticina* from durum wheat (Huerta-Espino and Roelfs, 1992, Ordoñez and Kolmer, 2007) showed the same results as those found by Ezzahiri *et al*. (1994) in Morocco during 1985, 1988, 1990 and 1992. The reason why the virulence frequency of durum and common wheat isolates became significantly equal to that of the set of differentials in the period 2005–2006 may have been that the *P* genes on isolates from common wheat

Table 1. Virulence phenotypes of *Puccinia recondita* isolates collected from durum and common wheat in Abda-Doukkala, Chaouia-Tadla, Gharb-Saïss, and Tangérois[a].

Gilmour code [a]	No. of isolates	Virulence to *Lr* genes	Collections
0000000	21	-	Abda-Doukkala,Chaouia-Tadla, Gharb-Saïss, Tangérois
0000044	2	23, 26	Abda-Doukkala, Chaouia-Tadla
0000200	2	18	Abda-Doukkala, Gharb-Saïss
0000302	1	17, 18, 25	Abda-Doukkala
0000400	2	20	Abda-Doukkala
0000742	1	17, 18, 20, 23, 25	Abda-Doukkala
0001100	1	14a, 17	Abda-Doukkala
0001102	1	14a, 17, 25	Abda-Doukkala
0001622	1	14a, 18, 20, 22, 25	Abda-Doukkala
0004504	1	15, 17, 20, 26	Abda-Doukkala
0005510	1	14a, 15, 17, 20, 21	Abda-Doukkala
0005732	1	14a, 15, 17, 18, 20, 21, 22, 25	Abda-Doukkala
0007300	1	14a, 14b, 15, 17, 18	Abda-Doukkala
0041320	1	10, 14a, 17, 18, 22	Abda-Doukkala
0041722	1	10, 14a, 17, 18, 20, 22, 25	Abda-Doukkala
0042655	1	10, 14b, 18, 20, 21, 23, 24, 26	Abda-Doukkala
0043220	1	10, 14a, 14b, 18, 22	Abda-Doukkala
0043360	1	10, 14a, 14b, 17, 18, 22, 23	Abda-Doukkala
0047362	1	10, 14a, 14b, 15, 17, 18, 22, 23, 25	Abda-Doukkala
0047762	1	10, 14a, 14b, 15, 17, 18, 20, 22, 23, 25	Abda-Doukkala
0057774	1	3ka, 10, 14a, 14b, 15, 17, 18, 20, 21, 22, 23, 26	Abda-Doukkala
0240760	1	3, 10, 17, 18, 20, 22, 23	Abda-Doukkala
0645562	1	3, 3bg, 10, 14a, 15, 17, 20, 22, 23, 25	Abda-Doukkala
1000041	1	1, 23, 24	Abda-Doukkala
1001000	2	1, 14a	Abda-Doukkala, Gharb-Saïss
1002712	1	1, 14b, 17, 18, 20, 21, 25	Abda-Doukkala
1041000	1	1, 10, 14a	Abda-Doukkala
1047776	1	1, 10, 14a, 14b, 15, 17, 18, 20, 21, 22, 23, 25, 26	Abda-Doukkala
1067720	1	1, 9, 10, 14a, 14b, 15, 17, 18, 20, 22	Abda-Doukkala
1202320	1	1, 3, 14b, 17, 18, 22	Abda-Doukkala
1244166	1	1, 3, 10, 15, 17, 22, 23, 25, 26	Abda-Doukkala
1247701	1	1, 3, 10, 14a, 14b, 15, 17, 18, 20, 24	Abda-Doukkala
1247767	1	1, 3, 10, 14a, 14b, 15, 17, 18, 20 22, 23, 24, 25, 26	Abda-Doukkala
5203717	1	1, 2b, 3, 14a, 14b, 17, 18, 20, 21, 24, 25, 26	Abda-Doukkala
5215700	1	1, 2b, 3, 3ka, 14a, 15, 17, 18, 20	Abda-Doukkala
0000011	1	21, 24	Abda-Doukkala
0000462	1	20, 22, 23, 25	Abda-Doukkala
0000620	1	18, 20, 22	Abda-Doukkala
0005102	1	14a, 15, 17, 25	Abda-Doukkala
0005362	1	14a, 15, 17, 18, 22, 23, 25	Abda-Doukkala
0006760	1	14b, 15, 17, 18, 20, 22, 23	Abda-Doukkala

(continued on the next page)

Table 1. *(continued from preceding page)*

Gilmour code [a]	Number of isolates	Virulence to *Lr* genes	Collections
0201762	1	3, 14a, 17, 18, 20, 22, 23, 25	Abda-Doukkala
0240242	1	3, 10, 18, 23, 25	Abda-Doukkala
0246502	1	3, 10, 14b, 15, 17, 20, 25	Abda-Doukkala
0402354	1	3bg, 14b, 17, 18, 21, 23, 26	Abda-Doukkala
1043460	1	1, 10, 14a, 14b, 20, 22, 23	Abda-Doukkala
1046602	1	1, 10, 14b, 15, 18, 20, 25	Abda-Doukkala
1047702	1	1, 10, 14a, 14b, 15, 17, 18, 20, 25	Abda-Doukkala
1245744	1	1, 3, 10, 14a, 15, 17, 18, 20, 23, 26	Abda-Doukkala
1245774	1	1, 3, 10, 14a, 15, 17, 18, 20, 21, 22, 23, 26	Abda-Doukkala
1605320	1	1, 3, 3bg, 14a, 15, 17, 18, 22	Abda-Doukkala
1400576	1	1, 3bg, 17, 20, 21, 22, 23, 25, 26	Abda-Doukkala
0000020	2	22	Gharb-Saïss
0000064	1	22, 23, 26	Gharb-Saïss
0000141	1	17, 23, 24	Gharb-Saïss
0000232	1	18, 21, 22, 23	Gharb-Saïss
0000421	1	20, 22, 24	Gharb-Saïss
0000542	1	17, 20, 23, 25	Gharb-Saïss
0000620	1	18, 20, 22	Gharb-Saïss
0000700	1	17, 18, 20	Gharb-Saïss
0010304	1	3ka, 17, 18, 26	Gharb-Saïss
0026377	1	9, 14b, 15, 17, 18, 21, 22, 23, 24, 25, 26	Gharb-Saïss
0040000	1	10	Gharb-Saïss
0040004	1	10, 26	Gharb-Saïss
0041000	1	10, 14a	Gharb-Saïss
0041662	1	10, 14a, 18, 20, 22, 23, 25	Gharb-Saïss
0043000	1	10, 14a, 14b	Gharb-Saïss
0053672	1	3ka, 10, 14a, 14b, 18, 20, 21, 22, 23, 25	Gharb-Saïss
0405044	1	3bg, 14a, 15, 23, 26	Gharb-Saïss
0410410	1	3bg, 3ka, 20, 21	Gharb-Saïss
1041002	1	1, 10, 14a, 25	Gharb-Saïss
0000306	1	17, 18, 25, 26	Tangérois
0001462	1	14a, 20, 22, 23, 25	Tangérois
0003000	1	14a, 14b	Tangérois
0040062	1	10, 22, 23, 25	Tangérois
0041040	1	10, 14a, 23,	Tangérois
0101561	1	2c, 14a, 17, 20, 22, 23, 24	Tangérois

[a] Seven-digit code based on a differential set of 21-*Lr* genes: *Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr3bg, Lr3ka, Lr9, Lr10, Lr14a, Lr14b, Lr15, Lr17, Lr18, Lr20, Lr21, Lr22, Lr23, Lr24, Lr25, and Lr26*.

Table 2. Virulence frequencies of *Puccinia recondita* isolates with regard to the type of wheat and the areas from which collections were made in Morocco during 2005 and 2006.

Gene	Virulence frequencies					
	Isolates from common wheat	Isolates from durum wheat	Collection of Gharb-Saïss	Collection of Abda-Doukkala	Collection of Tangérois	Collection of Chaouia-Tadla
Lr1	25	18	10	27	9.1	33
Lr2a	0	0	0	0	0	0
Lr2b	1.8	2	0	4.5	0	0
Lr2c	0	2	0	0	9.1	0
Lr3	20	6	0	18	0	29
Lr3bg	5.5	6	6.9	2.3	0	14
Lr3ka	1.8	8	10	4.5	0	0
Lr9	1.8	2	3.4	2.3	0	0
Lr10	29	32	24	36	18	33
Lr14a	31	48	24	50	36	38
Lr14b	25	20	10	32	9.1	29
Lr15	29	18	6.9	32	0	43
Lr17	44	38	17	57	18	52
Lr18	49	32	28	52	9.1	52
Lr20	38	40	28	45	18	52
Lr21	13	16	14	16	0	19
Lr22	42	28	31	36	27	43
Lr23	33	34	24	30	36	52
Lr24	9.1	10	10	11	9.1	4.8
Lr25	27	34	21	32	27	43
Lr26	18	18	17	18	9.1	24

Table 3. Shannon and Kosman measures of phenotypic diversity of four collections of *Puccinia recondita* in Morocco during 2005 and 2006.

Collection	Kosman	Shannon
Abda-Doukkala	0.443	0.683
Chaouia-Tadla	0.441	0.607
Gharb-Saïss	0.257	0.633
Tangérois	0.232	0.407

Table 4. Kosman measures of distance between virulence phenotypes of four collections of *Puccinia recondita* in Morocco during 2005 and 2006.

Collection	Kosman measure of distance			
	Abda-doukkala	Chaouia-Tadla	Gharb-Saïss	Tangérois
Abda-doukkala	***			
Chaouia-Tadla	0.158	***		
Gharb-Saïss	0.169	0.192	***	
Tangérois	0.177	0.196	0.115	***

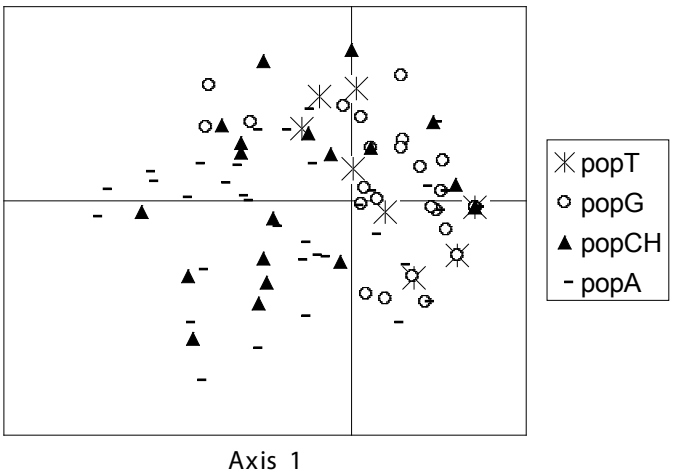

Figure 1. Principal coordinates analysis based on virulence data of 105 isolates of *Puccinia recondita.* Pop T: collection of Tangérois, Pop G: collection of Gharb-Saïss, Pop Ch: collection of Chaouia-Tadla, Pop A: collection of Abda-Doukkala.

became associated with fitness genes. Genes for fitness of course include genes for specific pathogenicity, permitting a wider host range, and this may have occurred in our study. Fitness genes could also have included other genes affecting survival or reproductive capacity (Johnson, 1987). The new fitness gained by common virulence phenotypes was expressed as a high degree of competitiveness against former durum virulence phenotypes.

The resistance genes *Lr2a*, *Lr2b*, *Lr2c*, *Lr3bg*, *Lr3ka*, *Lr9*, *Lr21*, *Lr24*, and *Lr26* gave good resistance against the collections of *P. recondita* from Abda-Doukkala, Chaouia-Tadla, Gharb-Saïss, and Tangérois. These genes may be rare or lacking in commercial Moroccan cultivars. This hypothesis cannot be verified since the *Lr* genes that are carried by the Moroccan cultivars are still unknown.

In 1998, 100% of *P. recondita* isolates were virulent to *Lr10* (Ezzahiri, 2000) but virulence frequency to this gene declined to only 10% in the two years of the study. The virulence frequency of *P. recondita* isolates to *Lr10* was found to be high in Israel (Maniterski *et al*., 2000), and the virulence frequency of *P. triticina* isolates in the United States, Spain, Mexico, France, Ethiopia, Chile and Argentina were also found to be high (Ordoñez and Kolmer, 2007). This decline in the virulence frequency of *P. recondita* to *Lr10* in Moroccan collections may be due to the high cost of excess of virulence (Leonard, 1977) which Vanderplank (1963, 1968) postulated as causing the reduced fitness of virulent genotypes. The decline in the virulence frequency to *Lr10* may also have been due to the introduction of Moroccan cultivars that lack this gene.

This is what Vanderplank (1963, 1968) called stabilizing selection, a selection force supposed to cause a decline of virulent genotypes when the corresponding genes on the host are lacking.

The ratio of the number of identified virulence phenotypes to the total number of isolates was 0.84, which indicated that the *P. recondita* population in Morocco was highly diverse and lacked a predominant virulence phenotype. This high virulence diversity could be a sign of frequent sexual recombinations (Felsenstein, 1996). Ezzahiri *et al*. (1992) found that the sexual reproduction of *P. recondita* in Morocco was generated from the alternate host *Anchusa italica*, which is common in most of the major Moroccan cereal-growing areas. The amalgamation of hyphae from different pathotypes may also lead to the formation of new pathotypes; this somatic hybridization was noticed in yellow rust, black rust and wheat leaf rust in wheat (Knott, 1989; Park and Felsenstein, 1998).

The collections from Abda-Doukkala and Chaouia-Tadla showed higher mean virulence complexities than the collections from Gharb-Saïss and Tangérois. This may have been due to the deployment of different resistance genes in the wheat cultivars grown in the last two areas. Østergärd and Hovmøller (1991) modelled associations between virulences on powdery mildew of barley with various arrangements of host resistance genes. Their models predicted that hosts with two or more combinations of resistance genes select complex pathogen phenotypes, whereas host populations with different resistance genes in different lines select simpler phenotypes. If directional selection resulted in only a few complex virulence phenotypes in the collection, then it would be expected that diversity would decrease (Kolmer, 1999). The Shannon and Kosman indices showed that the collection from Abda-Doukkala was the most diverse, whereas the collection from Tangérois was the least diverse. The low number of isolates from Tangérois explains this low diversity, since Kolmer *et al*. (2003) showed that sample size affected the diversity in wheat leaf rust populations. Using the Shannon index, we found that the collection from Gharb-Saïss was more diverse than the collection from Chaouia-Tadla. This result was expected since the greater number of virulence phenotypes in Gharb-Saïss increased the Shannon index, which considers each phenotype equally without regard to differences in virulence between phenotypes (Kolmer *et al*. 2003). With the Kosman index, the collection from Chaouia-Tadla had a higher diversity than the collection from Gharb-Saïss. This index takes into account differences in virulence between phenotypes as well as the frequencies of the phenotypes (Kosman, 1996).

With the Kosman distance index, the collections from Gharb-Saïss and Tangérois differed the least, and those from Abda-Doukkala and Chaouia-Tadla were the next least diverse. This finding may be explained by the geographical closeness of Abda-Doukkala and Chaouia-Tadla, while Gharb-Saïss is geographically close to Tangérois. The PCoA also confirmed the Kosman distance index, as isolates from Gharb-Saïss and Tangérois were the most closely related to each other. It can thus be concluded that the phenotypic diversity of *P. recondita* in Morocco is dependent on its geographical origin. This is confirmed by an earlier study in which isolates of *P. recondita,* collected from the same areas and during the same period, were examined by amplified fragment length polymorphism (AFLP) (Bouftass *et al*., 2009).

This study shows the high diversity of the Moroccan collections of *P. recondita* and the lack of a predominant virulence phenotype, which makes the breeding of wheat with leaf rust resistance in Morocco a very complicated undertaking. Further studies on resistance genes carried by Moroccan commercial cultivars should be done in order to prevent and anticipate shifts in virulence and virulence phenotypes of Moroccan collections of *P. recondita*.

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