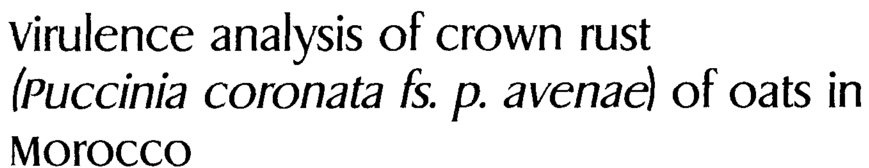

Virulence analysis of crown rust (*Puccinia coronata fs. p. avenae*) of oats in Morocco

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Abstract

The virulence of P. coronata f. sp. avenae isolates, collected from three regions of Morocco (Tangier, Gharb, and Doukkala) was determinated on out 23 differential lines and varieties of oat (Avena sativa), known to carry single gene for resistance. The patterns of virulence frequencies on the 23 differentials used were not different among the three regions, which indicate that the same pathogen population is present in these regions. In the Tangier, 55 virulence combinations or phenotypes were identified among 62 isolates studied; 83 were identified in the Gharb among 95 isolates, and 39 were identified among 40 isolates in the Doukkala. Virulence genes are randomly distributed among isolates, averaging 4.3 virulence genes in the Tangier, 4.8 in the Doukkala, and 5 in the Gharb. In the Doukkala and Tangier regions, the genes of virulence are randomly associated in the different isolates, while in the Gharb, some associations with more than three genes of virulence were more frequent than others.

Key words : Biotypes, crown rust, oat, virulence.

ملخص

لقد تم تعريف مجموعة من العينات الفطرية المسببة للصدأ التاجي *P. coronata f. sp. avenae* المنحدرة من مناطق الغرب، دكالة وطنجة. وقد بينت النتائج على ان هذه المناطق تحتوي على ساكنة واحدة من هذا الفطر. وهكذا تم تعريف 55 نوع من بين 62 عينة المنحدرة من دكالة و 83 عينة من بين 95 نوع منحدر من الغرب. وقد تبين كذلك أن جنايات الحدة تتوزع بين العشائر بطريقة عشوائية و هذا ما يدل على أن تكاثر هذا الفطر يمر عن طريق التوالد الجنسي.

الكلمات المفتاحية : عشيرة - الصدأ التاجي – الحدة– الشوفان

Résumé

La virulence des isolats de P. coronata f. sp. avenae, collectés dans trois régions du Maroc (Tangrois, Gharb et Doukkala) a été déterminée sur 23 lignées et variétés différentielles d'avoine (Avena sativa), connues porter un seul gène de résistance. Les fréquences de virulence sur les 23 différentielles utilisées n'ont pas été différentes entre les régions, ce qui indique qu'il s'agit d'une même population du pathogène. Dans le Tangerois, 55 phénotypes de virulence ont été identifiées parmi les 62 isolats analysés ; 83 ont été identifiées au Gharb pari les 95 isolats analysés, et 39 ont été identifiées à Doukkala parmi les 40 isolats analysés. Les gènes de virulence ont été aléatoirement distribués entre les isolats, avec une moyenne de 4.3 gène s de virulence au Tangrois, 4.8 à Doukkala et 5 au Gharb. Dans la région de Dopukkala et le Gharb, les gènes de virulence sont aléatoirement associés dans les différents isolats. Par contre, dans la région du Gharb, des associations de plus de trois gènes de virulence ont été plus fréquentes que d'autres.

Mots clés : Biotypes, rouille couronnée, avoine, virulence

Introduction

Crown rust, caused by *Puccinia coronata* Corda f.sp. avenae Eriks, is a widely distributed disease of cultivated oat (*Avena sativa L.*) and its closely related species in Morocco. The heaviest infections are observed in northern regions where dry matter losses have been estimated to 60% of the yield potential [G. Jaritz, per. comm.]. The cost benefit ratio of chemical control of crown rust on oat is not economical. Except for seed production, the low economic return of this crop does not allow the support of fungicides cost [Chin et al., 1975; Prusky et al., 1981]. The use of resistant cultivars is the only efficient way to overcome this disease [Simons, 1985].

Specific or vertical resistance has largely been used against crown rust since the beginning of this century. Many genes of resistance have been successfully transferred to commercial varieties [Simons et al., 1979; Sebesta and Kuhn, 1990; Aung et al., 1996]. However, the use of varieties presenting this type of resistance is at the origin of the selection of new virulent stocks of the pathogen. As the stocks increase, the effectiveness of resistance decreases. Thus, the use of single genes of resistance proved to be insufficient for a durable protection of crop in USA for example [Browing, 1973]. Therefore, the introgression of several genes of resistance in different lines, and the use of these lines in multiline cultivars, or their pyramiding in a same genotype, is necessary in order to prolong the effectiveness of specific resistance (*Chong and Komer,* 1993).

In Morocco, wild oats constitute a significant component of natural vegetation. Some of these species, such as *A. sterilis* and *A. barbata*, are widespread weeds in cereal fields, and are omnipresent with high abundance indexes [Link and Mouch, 1984]. In the Gharb, Tangier, and the Doukkala, where crown rust prevails regularly, the spores are present throughout even in the summer on wild species. The frequent mild temperatures (15 with 25°C) and abundance of dew are the most favourable conditions for the development of this disease. The temperature increase starting February ensures the ideal conditions to trigger the disease development, which coincides with stem elongation of the cultivated species. By undergoing each year the attack of rust, natural populations of these species thus maintain a reservoir of inoculum for the cultivated species and influence the virulence spectrum of the stocks of *P. coronata.*

So far, the pathogen populations have not yet been studied in Morocco. It is not known whether these stocks are identical in these different regions, or not. The Tangier and the Ghrab regions receive more than 500mm of precipitation yearly. Besides, dew and fog are abundant (200 days/year), wish make rust appears in February. On the other hand, in Doukkala, the annual rainfall is about 350mm, and crown rust appears as soon as in December, much earlier than in other site. In all three regions, however rust could be present through the year in irrigated fields.

The alternate host, *Rhamnus lyciodes L.* on which *P. coronata* was identified, is also widespread in many regions of Morocco [Rieuf, 1971]; but its role in the disease epidiomology has not yet been proved. The mode of sexual reproduction of *P. coronata* has a strong effect on the evolution of the population virulence, which in turn may affect the stability of the host resistance (Oard and Simons, 1983). Crown rust hat not been well investigated in Morocco and the structure of the pathogen populations virulence is not yet known.

The objective of this study is to characterise the biotypes of *P. coronata f.*sp. *avenae*, prevailing in Morocco to compare their frequencies in different regions, and to study the association among the virulence genes in these biotypes.

Materials and methods

Sampling Diseases

Random samples of infected plants were collected along an itinerary established to cover the regions concerned by the study. The sampling distance were average each 15 to 20 km. Leaf samples were collected during the second week of April 1995, from either cultivated oat, or its wild relatives. For each sample, data including the severity of the symptoms, the host species when possible, the date and place of collection, were noted.

Spores collection

In the laboratory, each sample was prepared by sucking the uredospores of several pustules in one single collected gelatine capsule (size 00), by the use of a spore collector (Browder, 1971) and 0.5 ml of a non toxic mineral oil (Soltrol M 170) added to the capsule content (Rowell, 1957). These capsules were immediately kept under cold temperature (4°C) before the inoculation on the universal susceptible genotype, CV Markton. The plants were grown in plastic pots containing a mixture of peat (1/3) and sand (2/3).

Ten days old plants were inoculated using the method described by Browder (1971). Uredospores contained in the gelatin capsule were sprayed on leaves by an inoculator connected to the vacuum pomp's air outlet. After inoculation, pots were placed for 24h in an incubation chamber where a humidifier used to a saturate the air with humidity. The plants were then placed in a growth chamber with a temperature of 20 ± 2°c, and a lighting of 11.000 μE/m_/s (Philips MLR), with a 12h: 12h (dark: light) photoperiod.

Seven days after inoculation, one single pustule was isolated from the basal part of an inoculated leaf sheath. The rest of the leaf, as well as the other leaves were cut off to avoid spore mixtures, and the selected pustule continued its development on the leaf piece that stayed attached to the plant. Two weeks after inoculation, spores produced by the selected pustule were sucked in a capsule. These spores constitute hence, an isolate, which will be multiplied separately on the variety Markton, and used to inoculate the differentials.

Differential lines

The differentials used in this study are utilised world-wide. They belong to two groups (Table 1):

- The first group is composed of varieties carrying single resistance genes (Appler, Landhafer and Trispernia). These varieties are used as differentials in the USA [Simons, 1985].
- The second group composed of 20 isogenic lines produced from the variety Pendek. They are designated Pc35, Pc38, Pc39, Pc40, Pc45, Pc47, Pc48, Pc50, Pc54, Pc55, Pc56, Pc58, Pc59, Pc60, Pc61, Pc62, Pc63, Pc64, Pc67, and Pc68 referring to the resistance gene they contained. These lines were used as differentials in Canada [Chong and Seaman, 1991]. All the resistance genes present in these differentials originated from A. sterilis.

Table 1: Differential lines used in determining the virulence genes of the isolates of *P. coronata f. sp. avenae* collected in 1995, in the Tangier, Gharb and Doukkala regions of Morocco.

Differential Genotypes		Gene for resistance
1	Appler	Pc1
2	Landhofer	Pc5
3	Trispernia	Pc6
4	Pc35	Pc35
5	Pc38	Pc38
6	Pc39	Pc39
7	Pc40	Pc40
8	Pc45	Pc45
9	Pc46	Pc46
10	Pc48	Pc48
11	Pc50	Pc50
12	Pc54	Pc54
13	Pc55	Pc55
14	Pc56	Pc56
15	Pc58	Pc58
16	Pc59	Pc59
17	Pc60	Pc60
18	Pc61	Pc61
19	Pc62	Pc62
20	Pc63	Pc63
21	Pc64	Pc64
22	Pc67	Pc67
23	Pc68	Pc68
Markton		None

Scoring of the seedling reactions

The reactions of the differential lines and varieties of oat were evaluated 14 days after inoculation. The scoring method used to evaluate these reactions is that adopted by Murphy (1935). The corresponding infection types were obtained according to the scale presented in table 2. The infection types 0 to 2 are classified in the resistant reaction category, while the infection types 3 and 4 correspond to susceptibility reactions. The host resistance corresponds to a non virulence of the isolate; and inversely, the host susceptibility translates the isolate virulence to that host.

Table 2.: Description of infection types used in evaluating the seedling reactions of differential lines inoculated with oat crown rust (Murphy, 1935).

Infection Type	Reactions
0	Immune: no pustules or other macroscopic symptomes of rust .
0	hyper susceptibility: presence of necrotic tissue
1	Very resistant: small pustules surrounded by necrotic tissue.
2	Moderate resistance: small pustules surrounded by chlorotic or necrotic tissue.
2+	Moderate resistance : medium size pustules bordered with necrotic tissue.
3	Susceptible: no necrosis, medium size pustules, with chlorotic tissue.
4	Very susceptible: large size pustules with no necrosis nor chlorosis.
X	Heterogeneous: pustules of variable sizes.

Data analysis

An inventory of virulence genes was made on the basis of the differential genotypes infected. The theory of the gene for gene relationship of Flor (1956) was used, while considering the virulence as recessive (Dinoor et al., 1988). According to this hypothesis, the virulence of an isolate reveals the presence of virulence genes in the homozygote recessive state. Thus, the susceptibility of a differential, carrying a known resistance gene, reveals the virulence gene corresponding to a specific pathogen isolate.

Phenotypes of the races were defined on the basis of the virulence formula of the isolates (avirulent/virulent). To make their comparison easier and facilitate the computation of their frequencies, races were given a specific order based on the increasing number of the different virulence genes they carry.

The virulence genes were analysed within each region by comparing the observed distribution to the Poisson one, using a k^2 test. If the gene distribution among the races follow the Poisson distribution, it indicates that sexual reproduction contributes to the pathogen cycle, if not, the asexual reproductive mode dominates (Roelfs and Groth, 1980). The frequencies of 2 and 3 virulence genes associations were compared to the product of their individual frequencies. If the virulence genes are randomly combined, the frequency of the associations is equal to the product of the frequencies of individual genes, otherwise, a selection pressure action may be suspected.

Results

Inventory and geographic distribution of the virulence genes

One hundred and ninety seven samples were collected from the three regions surveyed (40 from Doukkala, 62 from the Tangier, and 95 from the Gharb). At last one of the isolates originated from the Tangier region was virulent on each of the differentials (except of Pc38, Pc39, and Pc56). Thus, the Tangier isolates has up 20 virulence genes. All the differentials have shown their susceptibility to at least one isolate originated from Gharb region, except for the Pc39 genotype, which was resistant to all of them. This indicates that 22 virulence genes are dispatched among the collected samples. The isolates originated from Doukkala region shown to be virulent on the 23 differentials, without exception. Thus, the isolates of this region share at least 23 virulence genes.

Frequencies of isolates virulence

The frequency of virulent isolates on each differential genotype was calculated in each region. The comparison of the virulence frequencies on the differentials, among the three regions, showed a significant difference only for the Pc56 genotype. This latter was resistant to all the Tangier isolates, but susceptible to the isolates from other regions. For the other differentials, the virulence frequencies are the same in all the regions.

On the basis of their abundance in all the regions, the genes can be classified into 3 groups (Figure 1). The first group is made of virulence genes corresponding to the Pc1, Pc5, and Pc6 that are generally present in about 60% of the isolates. The second group, was classified as intermediate as the genes were present in 10 to 40% of the samples, included virulent genes that correspond to the Pc35, Pc40, Pc45, Pc46, Pc48, Pc50, Pc54, Pc56, Pc58, Pc59, Pc61, Pc64, Pc67, and Pc68 lines. The last group is made of rare virulence genes (frequencies below 10%), and contains virulence genes corresponding to the Pc38, Pc39, Pc55, Pc60, Pc62, and Pc63 lines.

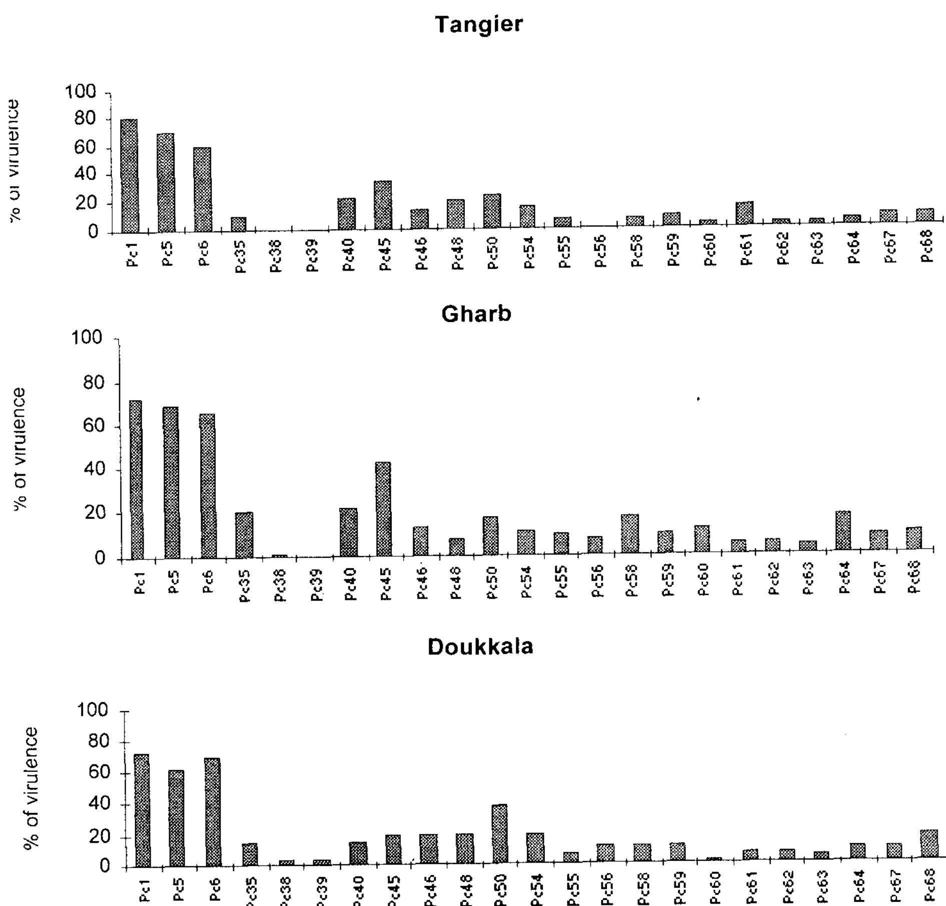

Figure 1: Frequencies of virulence in different isolates of *P. coronata f.sp. avenae* collected in the Tangier, the Gharb, and the Doukkala regions in 1995.

Identification of the isolates virulence

Tangier region

Among the 62 isolates studied from this region, 55 biotypes or phenotypes of virulence were identified. They are presented here in an increasing order of the number of virulence genes they carry (Table 3). The number of virulence genes by biotype varies from a minimum of one to a maximum of eight.

Table 3 : Combinations of the virulence genes of the isolates of *Puccinia coronata f.sp. avenae*, collected from wild and cultivated oat in the Tangier region in 1995.

Race	Nb of isolates	Virulent on lines
0	0	0
1	3	Pc1
2	1	Pc1, Pc5
3	1	Pc1, Pc6
4	1	Pc1, Pc45
5	1	Pc1, Pc46
6	1	Pc5, Pc6
7	1	Pc6, Pc48
8	1	Pc1, Pc5, Pc6
9	2	Pc1, Pc5, Pc45
10	1	Pc1, Pc5, Pc54
11	2	Pc1, Pc48, Pc50
12	1	Pc5, Pc45, Pc58
13	1	Pc35, Pc45, Pc54
14	1	Pc5, Pc48, Pc61
15	1	Pc1, Pc6, Pc40, Pc46
16	1	Pc1, Pc5, Pc35, Pc40
17	2	Pc1, Pc5, Pc6, Pc35
18	1	Pc1, Pc5, Pc6, Pc45
19	2	Pc1, Pc5, Pc6, Pc54
20	1	Pc1, Pc5, Pc6, Pc68
21	1	Pc5, Pc6, Pc45, Pc46
22	1	Pc5, Pc6, Pc59, Pc67
23	1	Pc5, Pc58, Pc62, Pc68
24	1	Pc40, Pc45, Pc61, Pc64
25	1	Pc1, Pc5, Pc6, Pc45, Pc46
26	1	Pc1, Pc5, Pc45, Pc50, Pc61
27	1	Pc1, Pc5, Pc45, Pc48, Pc61
28	1	Pc1, Pc6, Pc40, Pc59, Pc62
29	1	Pc1, Pc5, Pc6, Pc35, Pc54
30	1	Pc1, Pc5, Pc45, Pc58, Pc61
31	2	Pc1, Pc5, Pc6, Pc50, Pc54
32	1	Pc1, Pc5, Pc40, Pc45, Pc68
33	1	Pc1, Pc5, Pc45, Pc50, Pc61
34	1	Pc5, Pc6, Pc48, Pc62, Pc67
35	1	Pc1, Pc5, Pc46, Pc50, Pc61
36	1	Pc1, Pc5, Pc45, Pc50, Pc61
37	1	Pc1, Pc5, Pc6, Pc54, Pc55, Pc61
38	1	Pc5, Pc6, Pc40, Pc48, Pc55, Pc67
39	1	Pc1, Pc6, Pc35, Pc40, Pc46, Pc50
40	1	Pc1, Pc5, Pc6, Pc35, Pc46, Pc48
41	1	Pc1, Pc6, Pc35, Pc40, Pc45, Pc48
42	1	Pc1, Pc5, Pc6, Pc35, Pc45, Pc50
43	1	Pc1, Pc5, Pc6, Pc45, Pc48, Pc50
44	1	Pc1, Pc5, Pc6, Pc45, Pc50, Pc54
45	1	Pc1, Pc6, Pc35, Pc40, Pc50, Pc55
46	1	Pc1, Pc5, Pc6, Pc45, Pc50, Pc60
48	1	Pc1, Pc6, Pc46, Pc48, Pc55, Pc63
49	1	Pc1, Pc5, Pc6, Pc40, Pc48, Pc54, Pc59
50	1	Pc1, Pc5, Pc6, Pc40, Pc48, Pc64, Pc67
51	1	Pc1, Pc6, Pc40, Pc46, Pc50, Pc55, Pc63
52	1	Pc5, Pc6, Pc40, Pc50, Pc58, Pc60, Pc64
53	1	Pc5, Pc6, Pc40, Pc48, Pc58, Pc61, Pc68
54	1	Pc1, Pc5, Pc6, Pc40, Pc45, Pc50, Pc59, Pc67
55	1	Pc1, Pc5, Pc6, Pc46, Pc50, Pc54, Pc59, Pc67

Gharb region

In this region, 83 different biotypes were identified among the 95 isolates tested (Table 4). The highest number of virulence genes found in any isolates was twelve.

Table 4 : Combinations of the virulence genes of the isolates of *Puccinia coronata* f.sp. avenae, collected from wild and cultivated oat in the Gharb region in 1995.

Race	Nb of isolates	Virulent on lines
0	0	0
1	1	Pc1
2	1	Pc5
3	1	Pc6
4	1	Pc59
5	1	Pc1, Pc5
6	1	Pc1, Pc6
7	1	Pc1, Pc45
8	1	Pc1, Pc59
9	2	Pc5, Pc6
10	1	Pc6, Pc40
11	1	Pc6, Pc45
12	1	Pc6, Pc60
13	1	Pc35, Pc45
14	3	Pc1, Pc5, Pc6
15	2	Pc5, Pc6, Pc45
16	1	Pc1, Pc5, Pc61
17	2	Pc1, Pc45, Pc48
18	1	Pc5, Pc6, Pc59
19	2	Pc5, Pc40, Pc45
20	1	Pc5, Pc46, Pc62
21	1	Pc5, Pc50, Pc64
22	1	Pc6, Pc46, Pc48
23	4	Pc1, Pc5, Pc6, Pc45
24	1	Pc1, Pc5, Pc40, Pc60
25	1	Pc1, Pc5, Pc6, Pc63
26	1	Pc1, Pc5, Pc6, Pc67
27	1	Pc1, Pc5, Pc45, Pc56
28	1	Pc1, Pc5, Pc46, Pc59
29	1	Pc1, Pc5, Pc50, Pc54
30	1	Pc1, Pc5, Pc48, Pc63
31	1	Pc1, Pc6, Pc55, Pc58
32	1	Pc1, Pc6, Pc58, Pc64
33	1	Pc1, Pc6, Pc60, Pc61
34	1	Pc1, Pc6, Pc50, Pc67
35	1	Pc5, Pc6, Pc56, Pc60
36	1	Pc35, Pc45, Pc46, Pc58
37	2	Pc1, Pc5, Pc6, Pc40, Pc45
38	1	Pc1, Pc5, Pc6, Pc40, Pc50
39	1	Pc1, Pc5, Pc6, Pc45, Pc46
40	1	Pc1, Pc5, Pc6, Pc54, Pc61
41	2	Pc1, Pc5, Pc6, Pc56, Pc67
42	1	Pc1, Pc5, Pc40, Pc45, Pc67
43	1	Pc1, Pc50, Pc54, Pc56, Pc60
44	1	Pc5, Pc6, Pc35, Pc56, Pc68
45	2	Pc5, Pc6, Pc40, Pc58, Pc68
46	1	Pc5, Pc6, Pc45, Pc46, Pc48
47	1	Pc5, Pc6, Pc54, Pc55, Pc68
48	1	Pc1, Pc5, Pc6, Pc35, Pc45, Pc67
49	1	Pc1, Pc5, Pc6, Pc40, Pc45, Pc50
50	1	Pc1. Pc5. Pc6. Pc45. Pc54. Pc60
51	1	Pc1, Pc5, Pc6, Pc54, Pc55, Pc61
52	1	Pc1, Pc5, Pc6, Pc46, Pc50, Pc56
53	1	Pc1, Pc5, Pc6, Pc48, Pc56, Pc63
54	1	Pc1, Pc5, Pc6, Pc35, Pc50, Pc58
55	1	Pc1, Pc5, Pc6, Pc48, Pc56, Pc63
56	1	Pc1, Pc5, Pc6, Pc54, Pc55, Pc56
57	1	Pc1, Pc5, Pc35, Pc45, Pc48, Pc67
58	1	Pc1, Pc5, Pc40, Pc56, Pc62, Pc67
59	1	Pc1, Pc6, Pc35, Pc40, Pc45, Pc59
60	1	Pc1, Pc6, Pc56, Pc59, Pc64, Pc68
61	1	Pc1, Pc35, Pc45, Pc46, Pc55, Pc58
62	1	Pc1, Pc40, Pc45, Pc60, Pc67, Pc68
63	1	Pc1, Pc5, Pc6, Pc45, Pc50, Pc54, Pc55
64	1	Pc1, Pc5, Pc35, Pc54, Pc55, Pc58, Pc63
65	1	Pc1, Pc5, Pc55, Pc58, Pc60, Pc61, Pc68
66	1	Pc5, Pc6, Pc35, Pc55, Pc58, Pc67, Pc68
67	1	Pc5, Pc6, Pc40, P50, Pc45, Pc58, Pc68
68	1	Pc5, Pc6, Pc35, Pc55, Pc56, Pc59, Pc62
69	1	Pc1, Pc5, Pc35, Pc45, Pc50, Pc64, Pc67
70	1	Pc1, Pc5, Pc6, Pc45, Pc48, Pc50, Pc61
71	1	Pc1, Pc5, Pc6, Pc40, Pc59, Pc62, Pc64
72	1	Pc1, Pc5, Pc6, Pc45, Pc50, Pc54, Pc60, Pc64
73	1	Pc1, Pc5, Pc40, Pc45, Pc54, Pc58, Pc64, Pc67
74	1	Pc1, Pc5, Pc6, Pc46, Pc54, Pc56, Pc58, Pc59
75	1	Pc1, Pc5, Pc6, Pc35, Pc54, Pc55, Pc56, Pc59, Pc64
76	1	Pc1, Pc5, Pc6, Pc45, Pc58, Pc60, Pc64, Pc67, Pc68
77	1	Pc5, Pc6, Pc35, Pc45, Pc54, Pc55, Pc59, Pc67, Pc68
78	1	Pc1, Pc6, Pc35, Pc45, Pc46, Pc48, Pc58, Pc59, Pc64, Pc67
79	2	Pc1, Pc5, Pc6, Pc45, Pc46, Pc50, Pc58, Pc62, Pc64, Pc67
80	1	Pc1, Pc5, Pc6, Pc45, Pc46, Pc50, Pc59, Pc60, Pc62, Pc67
81	1	Pc1, Pc6, Pc35, Pc45, Pc46, Pc48, Pc50, Pc60, Pc61, Pc67
82	1	Pc1,Pc5,Pc6,Pc35,Pc45,Pc46,Pc54,Pc59,Pc62,Pc64,Pc68
83	1	Pc1,Pc5,Pc35,Pc40,Pc45,Pc46,Pc50,Pc54,Pc56,Pc58, Pc60 Pc67

Doukkala region

In the Doukkala region, out of the 40 isolates analysed, 39 different biotypes were identified (Table 5). Except for one case, all the identified combinations were represented by only one isolate. The isolates carrying the largest number of virulence genes had up to nine.

Table 5 : Combinations of the virulence genes of the isolates of *Puccinia coronata f.sp. avenae*, collected from wild and cultivated oat in the Doukkala region in 1995.

Race	Nb of isolates	Virulent on the lines
0	0	0
1	1	Pcl
2	1	Pcl, Pc5
3	1	Pc6, Pc60
4	1	Pcl, Pc5, Pc67
5	1	Pcl, Pc6, Pc45
6	1	Pc5, Pc6, Pc54
7	1	Pcl, Pc46, Pc58
8	1	Pc5, Pc6, Pc39
9	1	Pc5, Pc6, Pc58
10	1	Pc5, Pc60, Pc68
11	1	Pc6, Pc50, Pc54
12	1	Pcl, Pc5, Pc6, Pc40
13	1	Pcl, Pc5, Pc6, Pc46
14	1	Pcl, Pc5, Pc64, Pc68
15	1	Pcl, Pc6, Pc40, Pc50
16	1	Pcl, P5, Pc58, Pc60
17	1	Pcl, Pc40, Pc45, Pc46
18	1	Pc5, Pc6, Pc48, Pc50
19	1	Pc5, Pc6, Pc45, Pc50
20	1	Pc48, Pc50, Pc60, Pc64
21	1	Pcl, Pc5, Pc6, Pc50, Pc54
22	1	Pcl, Pc5, Pc6, Pc48, Pc59
23	1	Pcl, Pc5, Pc35, Pc55, Pc64
24	1	Pcl, Pc45, Pc48, Pc50, Pc56
25	1	Pcl, Pc5, Pc35, Pc45, Pc58
26	2	Pcl, Pc5, Pc6, Pc58, Pc50
27	1	Pc5, Pc6, Pc46, Pc59, Pc62
28	1	Pcl, Pc5, Pc6, Pc50, Pc54, Pc68
29	1	Pcl, Pc5, Pc6, Pc55, Pc59, Pc62
30	1	Pcl, Pc5, Pc6, Pc58, Pc67, Pc68
31	1	Pc6, Pc45, Pc46, Pc48, Pc50, Pc62
32	1	Pc6, Pc55, Pc58, Pc63, Pc67, Pc68
33	1	Pcl, Pc5, Pc6, Pc35, Pc38, Pc50, Pc54
34	1	Pcl, Pc6, Pc35, Pc48, Pc63, Pc67, Pc68
35	1	Pcl, Pc35, Pc45, Pc46, Pc48, Pc60, Pc61
36	1	Pcl, Pc5, Pc6, Pc35, Pc40, Pc45, Pc46, Pc50
37	1	Pcl, Pc5, Pc6, Pc40, Pc45, Pc50, Pc62, Pc64
38	1	Pcl, Pc5, Pc6, Pc40, Pc45, Pc48, Pc50, Pc59, Pc67
39	1	Pcl, Pc5, Pc6, Pc45, Pc46, Pc54, Pc56, Pc59, Pc68

Distribution of the virulence genes among the biotypes

The distribution of the frequency of the number of virulence genes present in the isolates was established for each of these surveyed regions. The differences between the observed and the theoretical values were not significant. The distributions of the frequency of the number of virulence genes among the isolates in the three regions fallow a Poisson distribution. Genes are randomly distributed among the isolates, with a mean of 4.3 genes in the Tangier; 4.8 in Doukkala; and 5 in the Gharb (Figure 2).

Association of the virulence genes

The frequencies of the 210 associations (in pairs and triplets) of virulence genes were analysed for each of the three regions. In the Tangier, of all the associations studied, only four virulence gene pairs showed a frequency significantly lower than the product of the frequencies of individual genes (Table 6). For the other pairs, the observed frequencies did not differ from the expected values. In the Gharb region, 13 pairs of virulence genes showed frequencies significantly different from the product of their individual genes frequencies. The Pc1-Pc63 and Pc1-Pc64 gene pairs had frequencies lower than the expected values, thus negatively associated. On the opposite, the 11 other pairs having frequencies higher than the expected values, were positively asso-

ciated. In the Doukkala region, no gene pair was found in association. Of the associations studied in the Doukkala and the Tangier regions, no triplet of virulence genes showed any significant difference between the observed and expected frequencies. In the Gharb region, 24 of the 210 expected combinations of 3 virulence genes had frequencies significantly higher than the product of their individual frequencies (Table 6).

Table 6 : Virulence genes of *P. coronata avenae* associated in pairs and triplets in isolates collected in 1995, from the three surveyed regions.

Region	Pairs of genes in association
Tangier	Pc1-Pc6, Pc6-Pc45, Pc6-Pc61, Pc45-Pc54
Gharb	Pc1-Pc63, Pc1-64, Pc6-Pc45, Pc6-Pc46, Pc35-Pc45, Pc35-Pc46, Pc35-Pc54, Pc35-Pc55, Pc35-56, Pc35-Pc58, Pc35-Pc59, Pc54-Pc55, Pc58-Pc68
Doukkala	-
	Triplets of genes in association
Tangier	-
Gharb	Pc1-Pc35-Pc45, Pc1-Pc35-Pc46, Pc1-Pc35-Pc54, Pc1-Pc35-Pc58 Pc1-Pc35-Pc67, Pc1-Pc40-Pc45, Pc1-Pc40-Pc67, Pc1-Pc45-Pc48 Pc1-Pc45-Pc58, Pc1-Pc45-Pc67, Pc1-Pc50-Pc54, Pc1-Pc50-Pc60 Pc1-Pc50-Pc64, Pc1-Pc54-Pc56, Pc1-Pc54-Pc58, Pc1-Pc54-Pc60 Pc1-Pc54-Pc64, Pc1-Pc55-Pc58, Pc1-Pc55-Pc68, Pc1-Pc56-Pc58 Pc1-Pc58-Pc64, Pc1-Pc58-Pc67, Pc1-Pc60-Pc67, Pc1-Pc64-Pc67
Doukkala	-

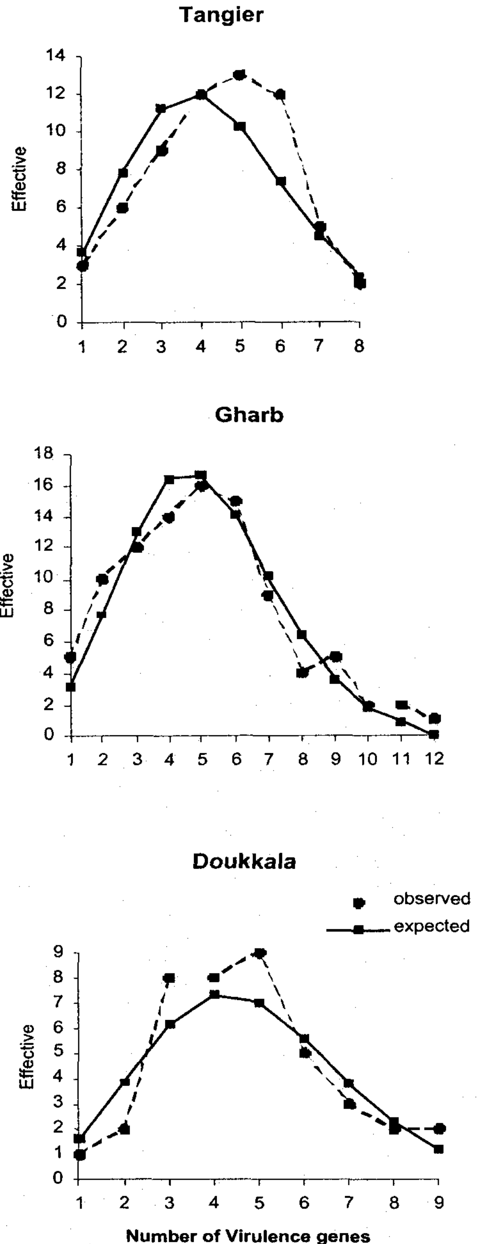

Figure 2: Distribution of the frequency of the number of virulence genes associations in the isolates of *P. coronata f.sp. avenae* collected in 1995 from the three geographic regions studied.

Discussion

The analysis of isolates from the three regions allowed the identification of 179 different virulence races or phenotypes among the 197 samples analysed. The most abundant phenotypes were being represented by a maximum of four isolates. The virulence genes corresponding to Pc1, Pc5, and Pc6 are present in more than 60% of the isolates. Those corresponding to Pc35, Pc40, Pc44, Pc45, Pc46, Pc48, Pc50, Pc54, Pc56, Pc58, Pc59, Pc61, Pc64, and Pc68 have a frequency between 10 and 40%. The virulence genes corresponding to Pc38, Pc39, Pc55, Pc60 and Pc63 are the

least common (frequency lower than 10%). This difference of frequency for some virulence genes could be explained by a selection pressure action that is favourable to them was the considering that the corresponding resistance genes have a higher frequency.

The ratio of the number of identified biotypes to the number of collected isolates (0.88 in the Tangier and the Gharb, and 0.97 in the Doukkala) translates a high level of the pathogen variability in these regions of Morocco. The absence of the dominance of a given race, reflects the adaptation of the pathogen to the largely diverse populations of the host, Avena spp. [Wahl, 1970]. In fact, species of the genus Avena, which are the primary host of crow rust, is an important component of spontaneous vegetation. Populations of these species are highly diverse; some of themes are common weeds in cereals. It is the case of A. barbata and A. sterilis, which are widely present, with high abundance indexes [Link and Mouch, 1984]. These species insure the infection by the pathogen the year around, and thus, contribute to the diversification and the preservation of the genetic variability of this latter. Dinoor and Eshed (1984) reported on a similar situation on rust in Israel.

The analysis of the frequencies of the associations of virulence genes did not show any significant difference between the expected and the observed values for the pairs and the triplets of virulence genes in the Doukkala region. In the Tangier region , out of the 210 associations studied, three cases of negative associations in pairs of virulence genes, were statistically significant ; and no case of three genes association. These results do not show any action of selection pressure. However, in the Gharb region 13 pairs of virulence genes showed frequencies significantly different from the expected values. Eleven pairs had frequencies higher than the product of their individual frequencies, and two had frequencies lower than the expected values. For the three gene associations, 24 triplets had frequencies significantly higher than the product of their individual frequencies.

The abundance of two and three gene associations (their individual frequencies vary from two to 30% for the pairs, and from two to 13% for the triplets) translates an action of selection pressure in favour of the biotypes carrying these virulence genes. Differences observed between the Gharb and other regions could be explained by the diversity of the hosts populations, but mainly by the climatic conditions that are generally favourable to the infection. In fact, in the Gharb plain, there are more than 200 days of dew and fog per year, which are favourable conditions for spore germination.

The distribution of the number of virulence genes in the isolates fallows a Poisson distribution. Sexual recombination most likely acts by a random distribution of these virulence genes during meiosis. The contribution of sexual reproduction in the pathogen cycle is an essential element to consider when elaborating a strategy of resistance gene deployment.

The inoculation test of the 23 monogenic differentials showed that for each gene for resistance, there is a matching virulence gene in the pathogen population. When varieties carrying resistance genes are in contact with these virulence genes in the pathogen, there is a risk of selection of new races that are virulent to these resistance genes, and thus a quicker break down of the resistance of these varieties. To avoid such situation, it is recommended to adopt a gene pyramiding or multiline variety strategy.

Complex races (carrying more than 3 virulence genes) are very abundant (70%) in the pathogen population as compared to the simple races (carrying one to three virulence genes). In these conditions, controlling rust by resistance genes pyramiding would not be recommended. The presence, in the pathogen population, of races carrying up to 12 virulence genes, could induce the rapid breakdown of the resistance of a variety caring any gene for resistance. Contrarily, using a multiline variety offers the possibility of replacing a line of

the mixture that becomes susceptible by a new resistant one. The multiline varieties have been widely and successfully used in the USA (Frey, 1982), and have been confirmed as a control method that is most adapted against genetically variable pathogens such as the rusts (Mundt and Leonard, 1986).

Prior to breeding multiline varieties, studies of the pathogen evolution, by applying adequate theoretical models to epidemiological data (Lannou and Mundt, 1996) need to be carried out. Results of simulating the pathogen evolution will allow a better knowledge of its behaviour, and help in directing experimental research concerning the choice of the resistance genes to be used in the lines, as well as their proportions in the mixture.

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