Genetic and virulence diversity in a Jordanian field population of *Rhynchosporium secalis* on barley

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Abstract

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Genetic structure of *R. secalis* field population hierarchically sampled in Jordan (Al-Rabba) was determined using AFLP markers. From a total of 35 isolates, 34 multilocus haplotypes were identified with overall genotypic diversity 95% of theoretical maximum. Majority of gene diversity (57%) was distributed within 1 m^2 sampling area in the field. Significant linkage disequilibrium and high genetic diversity suggest that the fungus may possess mechanisms for new variation in the absence of sexual reproduction. A subset of 17 isolates tested for virulence on a set of 17 cultivars, were grouped into 17 different pathotypes. The pathotypes were virulent on at least three of the differential cultivars and no differential was resistant to all pathotypes. Pathotype complexity ranged from 3 to 15, with mean complexity of 11. There was no significant association between AFLP and virulence attributes of the isolates. Our results indicated that the pathogen population in Jordan possesseed high genetic diversity and unnecessary virulence and thus the use of major resistance gene may not provide long-term protection under Jordanian conditions.

Key words: Genetic structure, AFLP, scald, Jordan.

Introduction

Barley scald, caused by *Rhynchosporium secalis* (Oud.) Davis is an economically important disease of barley worldwide and causes severe yield losses in highly susceptible cultivars (21). *R. secalis* has a reputation for extensive pathogenic variability and ability to generate new virulence phenotypes (8) through a range of mechanisms including spontaneous mutation (13, 16), frequency dependent selection (14) and asexual (31, 33) and sexual recombination (22).

Infected barley stubble and seeds have been reported to be the major sources of primary inoculum (4, 14, 15, 18) and wild grasses serve as additional sources of inoculum of the pathogen (14,18). In Jordan, barley scald has been widely distributed in most of the barley growing areas and has been isolated from both cultivated barley (*Hordeum vulgare* L.) and *H. spontaneum* C.Koch. In Australia, wild grasses play an important role in shaping the genetic structure of the scald population, by selectively favoring a broad range of pathogenicity (19) and were reported as sources of genetically diverse pathogenic variants of *R. secalis* (1, 2, 7). The pathogen population in Jordan is expected to be genetically and pathogenically diverse considering the fact that the country is in the region of the center of origin of the crop and presumably center of origin of the pathogen. Populations at the center of origin of a species generally have the greatest gene diversity (24) and the existence and wide distribution of the wild progenitor of cultivated barley, *H. spontaneum*, in Jordan could possibly contribute to the genetic and pathogenic diversity of the pathogen. However, information on the level of genetic and virulence diversity of the pathogen in Jordan is needed. Therefore, this study was conducted to measure the level of genetic and virulence diversity in *Rhynchosporium secalis* in Jordan by intensively sampling a single field of cultivated barley.

Materials and Methods

Field sampling method, isolation and culturing of R. secalis

Barley leaves infected with *R. secalis* were sampled from naturally infected fields of local cultivar of barley in Al-Rabba, Jordan using a hierarchical sampling method (27). Two transects approximately 10 m apart and four sampling stations of 1m diameter at 10 m interval along each transect were sampled. Ten infected leaves were sampled in a circular sweep from different plants at each of the eight 1m diameter circular sampling stations in each field during the 2003 crop season. Barley leaves containing typical scald lesions were placed in paper envelopes, air dried at room temperature for at least one week, and stored at 5 °C. Dried leaf pieces cut from scald lesions were surface sterilized by dipping in 90% ethanol for 10 seconds, followed by 60 seconds in 0.5% sodium hypochlorite solution. Wet pieces were press dried between two layers of sterile filter paper and placed on a plastic mesh that rested on rubber bands on top of damp sterile filter paper in a petri dish. The petri dishes were incubated in the dark at 16 °C for at least 72 hours to induce fungal growth and sporulation. Using a sterile inoculation loop, masses of conidia were streaked on to the surface of potato dextrose agar (PDA) medium and single germinating conidia were transferred into lima bean agar (LBA) plates amended with 10 mg/l of gentamycin-sulphate. After about two weeks of growth, single colonies were picked and macerated in a test tube containing sterile distilled water using a sterile glass rod. The resulting spore and mycelial suspension was transferred into 2 ml microfuge tubes and kept as a stock culture at –20 °C for long-term storage.

DNA extraction

Fourteen-day-old fungal cultures, grown on LBA medium, were scraped using sterile microscope slides and macerated in sterile test tubes containing sterile distilled water. The ensuing conidial and mycelial suspension was

used to inoculate 50 ml erlenmeyer containing 40 ml of potato dextrose broth amended with 20 mg/l of gentamycin-sulphate. Inoculated flasks were kept in the dark at 17±1 °C on an orbital shaker (150 rpm) for up to six weeks after which the fungal material was collected and lypholized for a minimum of 72 hours in a freeze drier. Approximately 0.05 g of freeze-dried fungal tissue was ground into fine powder with beads in 2 ml microfuge tubes using a mixer-mill (mixer-mill Retsch MM 2000). Fungal DNA was extracted using CTAB protocol following the method of von Korff *et al.* (42). The DNA concentration was estimated by comparing the intensity of ethidium bromide fluorescence of sample DNA to a known concentration of lambda DNA on agarose gel using a UV transilluminator imaging system (Eagle Eye, Stratagene, La Jolla, CA, USA). The concentration of extracted DNA was also quantified by taking spectrophotometric absorbance (A) readings at wavelengths (λ) of 260 nm.

AFLP analysis

Amplified fragment length polymorphism (AFLP) analysis was carried out by following the method of Vos *et al.* (43), with few modifications as described by von Korff *et al.* (42). Three pairs of primers that generated more distinct and relatively more polymorphic bands, M301 (TATA) - P24 (TC), M183 (CCCT)-P18 (CT) and M289 (TAAA)-P16 (CC), were selected for AFLP analysis after screening sixteen different *Tru*9I and *Pst*I primer combinations on a subset of eight randomly selected isolates of the fungus. To check for repeatability, a second AFLP analysis using the three primer combinations was conducted and AFLP data was recorded based only on distinct bands.

Data analysis

The AFLP fragments of distinct size were treated as markers at individual loci with two alleles encoding presence or absence of a band. The combined allelic-state of an isolate, at all considered AFLP loci, is designated as the multilocus haplotype (MLHT). The genotype diversity ($\hat{G}$) of a population was calculated according to Stoddart and Taylor (40). The maximum possible value for $\hat{G}$, which occurs when each individual in the sample is unique, is the number of individuals in the sample. $\hat{G}$ was expressed as the percentage of maximum diversity to compare the values of $\hat{G}$ for populations that differ in sample sizes.

Analysis of gene diversity was carried out in order to estimate the distribution of variability within and among subpopulations and population subdivisions as implemented by "POPGENE" (46) and following methods suggested by Nei (30). Each of the eight sampling stations within the field was considered as an individual subpopulation during the analysis. The total gene diversity (*Ht*) was partitioned into gene diversities among and within (*Hs*) subpopulations. Genetic differentiation (G_{ST}) of subpopulations relative to the total population was calculated using the coefficient of gene differentiation (28, 29). The amount of gene flow between subpopulations, *Nm*, where *N* is the effective population size and *m* is the fraction of individuals in a population that are migrants, was estimated using the formula of Boeger *et al.* (5). The Jaccard similarity matrix was used for cluster analysis (unweighted pair-group method with arithmetic mean; UPGMA) using the software package NTSYS-PC version 2.02 (36). The measure of multilocus associations was estimated using clone corrected data. Alleles with only intermediate frequency (> 0.1 and < 0.9) were considered for analysis.

Isolate pathotyping

Seventeen isolates of the fungus selected from a total of 36 isolates were tested for virulence using a set of 17 differential host cultivars (Table 1). Each of the eight sampling stations in the sampled field was represented by 2 or 3 isolates except in one station that was represented by a single isolate.

Each of the isolates were cultured on faba bean yeast extract agar plates in the dark at 16±1°C for 12 days after which the surface of the fungal culture was scraped using sterile glass microscope slides, homogenized in a household blender and the resulting suspension filtered through a fine plastic mesh to remove mycelial fragments and pieces of culture media. The suspension was centrifuged at 3000 rpm for 5 min, supernatant discarded and the pellet re-suspended in 50 ml sterile distilled water. A drop of Tween 20 (Fisher Biotech, New Jersey, U.S.A.) was applied into the conidial suspension. Inoculum, at a concentration of $1x10^6$ conidia/ ml, was used to inoculate ten-day-old plants. Inoculated plants were kept for 24 hours in a humidity chamber and were transferred into a growth chamber. A set of cultivars, sprayed with only sterile distilled water, was used as a check for extraneous air- or seed-borne inoculum. The seedlings of each cultivar-isolate combination were evaluated 17 days after inoculation on a five-point scale similar to that used by Ceoloni (9) with a slight modification as follows: 0 = no visible symptoms; 1 = small lesions confined to leaf tips and brown to gray necrotic spots; 2 = somewhat larger lesions; 3 = larger and coalescing lesions with distinct margins; and 4 = total collapse of the leaf with no discrete lesions within the wilted area. In accordance with Jackson and Webster (44), disease ratings 0, 1, and 2 were considered as resistant and 3 and 4 as susceptible.

Table 1. Differential cultivars and their respective resistance genes[1]

No.	Cultivar	C.I.[2]	Resistance genes
1	Armelle	-	Rh, BRR1
2	Astrix	-	BRR2
3	Athene	-	BRR3
4	Igri	-	BRR4
5	La-Mesita	-	Rh4, Rh10, Rh at Rh-Rh3-Rh4, BRR5
6	Osiris	1622	Rh4, rh6; Rh10; (Rh3?) BRR6
7	Pirate	-	BRR7
8	Digger	-	Partial resistance
9	Trebi	936	Rh4; rh6=rh?; Rh at Rh-Rh3-Rh4
10	Kitchin	1296	Rh9 (incomplete)
11	Steudelli	2226	rh6; rh7
12	Bey	5581	Rh3(?)
13	Atlas-46	7323	Rh; Rh2; Rh3
14	Modoc	7566	(Rh4); Rh2; rh6; (Rh3?); Rh at Rh-Rh3-Rh4
15	Forrajera	8158	Unknown
16	Abyssinian	668	(Rh1); Rh9
17	Rihane-3	local	unknown

[1] Accession number of the cereal crops Research Branch, A. R. S., U.S.D.A., Beltsville, Maryland;

[2] Table adapted from Pinnschmidt and Jakob Willas (www.crpmb.org/scald)

Results

The AFLP analysis resulted in a total of 78 bands, 82% of which were polymorphic. However, bands (alleles) with only intermediate frequency (> 0.1 and <0.9) were considered for the data analysis.

Comparison of population genetic structure of R. secalis

Based on the AFLP data, the 36 isolates were grouped into 35 multilocus haplotypes (MLHTs). The estimated overall genotypic diversity was 94.7% of the theoretical maximum with one MLHT represented by a pair of isolates that belong to two adjacent sampling stations that were 10 m apart.

The overall gene diversity estimated was 0.380 (±0.009) with 57% of the total gene variation distributed within the smallest spatial scale of 1 m^2 sampling station while 43% was distributed among sampling stations within the field.

Between paired sampling stations genetic distance (Table 2) ranged from 0.031 to 0.507 and population differentiation (G_{ST} = 0.4259) was relatively large and gene flow (*Nm*) was low (0.674). As shown in the phenogram (Figure 1), there was less pattern of clustering though the majority of the isolates that belong to three of the sampling stations (E, F, G) were in the same sub-branch while the second sub-branch was occupied by isolates from five of the sampling stations (B,C, D, F, and H) with one isolate from one sampling station (B) common to both subgroups. The multilocus index of association (I_A), used to estimate the level of multilocus linkage disequilibrium, was significantly high.

Variations in virulence in field populations

A subset of 17 isolates from a total of 36 isolates representing the *R. secalis* field population were examined for their virulence spectrum on 17 differential host cultivars (Tables 3 and 4). Based on the reaction of these differential host cultivars, 17 isolates were grouped into 17 different pathotypes. The pathotypes were named using a combination of the first letter of the name of the collection site and consecutive numbers. The term "pathotype" (41) is used in this study to denote *R. secalis* isolates that differed in virulence on the barley differentials tested. The pathotype complexity ranged from 3 to 15 with a mean complexity of 11. A complex pathotype is defined here as one capable of producing disease on a large number of differential strains of the host than a simple pathotype (44). Sixty-five per cent of the pathotypes were virulent on 13 to 15 of the differential host cultivars. From among the differentials, 'Kitchin', 'La-Mesita', and 'Forrajera' were the most susceptible cultivars and while 'Kitchin' was resistant to none of the pathotypes, 'La-Mesita', and 'Forrajera' were resistant to only one pathotype each. Cultivars 'Pirate' 'Atlas-46' and 'Rihane-3' were the most resistant ones and were susceptible to 2, 3 and 4 of the pathotypes, respectively.

Cluster analysis using virulence data, using both the 0 to 4 disease score data (Figure 2) and the host reaction data (resistant and susceptible) (Figure 3), showed no distinct pattern of clustering. Test for association between the pathogenic and AFLP diversity of the 17 selected isolates was done using the AFLP and differential host reaction data and no significant association between the virulence and AFLP diversity of the isolates was found.

Table 2. Estimated genetic distance between paired sub-populations in a field population of *Rhynchosporium secalis* sampled in Jordan (estimated based on Nei's unbiased measures of genetic identity and genetic distance (29).

Sub population[1]	A	B	C	D	E	F	G
A	0						
B	0.031	0					
C	0.092	0.065	0				
D	0.157	0.168	0.120	0			
E	0.279	0.268	0.424	0.429	0		
F	0.241	0.227	0.413	0.431	0.074	0	
G	0.397	0.365	0.507	0.342	0.044	0.048	0
H	0.214	0.185	0.146	0.143	0.342	0.291	0.278

[1] Sub-population refers to a group of isolates that belong to the same 1m^2 sampling-station in each field. Sampling was done on eight sampling stations of 1 m diameter along two parallel transects, four sampling stations per transect and distance between transects and between adjacent sampling stations was 10 m.

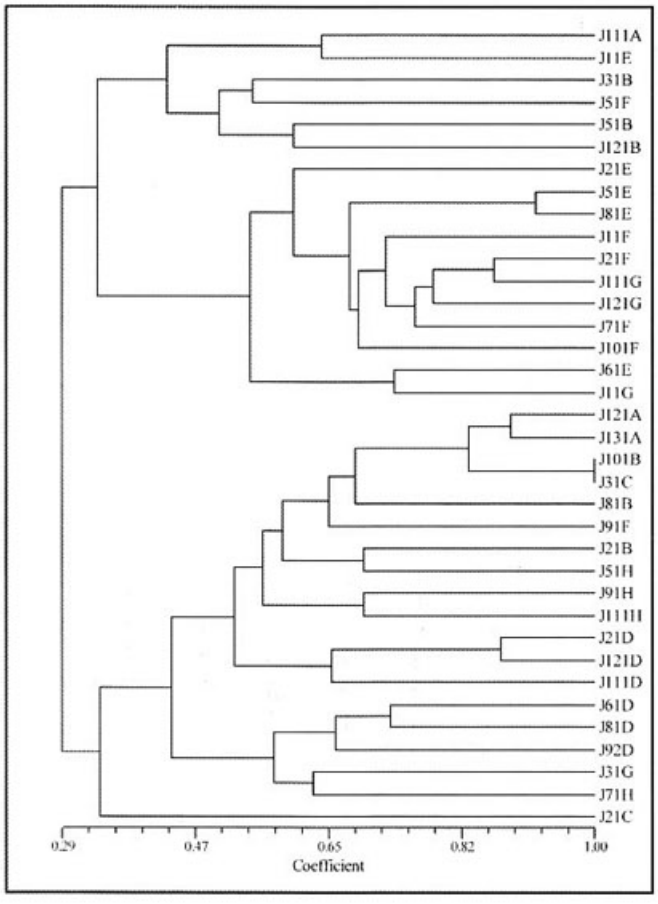

Figure 1. Phenogram of *Rhynchosporium secalis* isolates based on amplified fragment length polymorphic loci (AFLP) analysis. Jaccard's coefficient in the Numerical Taxonomy and Multivariate Analysis System for personal computers (NTSYS-pc; Exeter Software) generated distance matrix data of the AFLP data. The name of the isolates is coded: J stands for country of origin (Jordan), followed by isolate reference number in each sampling stations and letters 'A' to 'H' refering to individual sampling stations in the field.

Table 3. Reaction of 17 differential host cultivars to 17 Jordanian *R. secalis* isolates, and resulting pathotypes and their pathotype complexity and host gene complexity*.

Pathotype	Armelle	Astrix	Athene	Igri	La-Mesita	Osiris	Pirate	Digger	Trebi	Kitchin	Steudelli	Bey	Atlas-46	Modoc	Forrajera	Abyssinian	Rihane-3	Pathotype Complex
J01			S		S					S					S			4
J02			S	S	S					S		S		S	S			7
J03	S	S	S	S	S	S	S		S	S	S	S		S	S	S		14
J04	S		S	S	S			S	S	S	S	S		S	S	S	S	13
J05	S									S				S				3
J06	S		S	S	S	S			S	S		S		S	S	S		11
J07	S		S	S	S	S		S	S	S	S	S		S	S	S		13
J08	S	S	S	S	S	S			S	S	S	S		S	S	S		13
J09	S		S		S				S	S		S		S	S			8
J10	S	S	S	S	S		S	S	S	S	S	S	S	S	S		S	15
J11			S	S	S				S	S		S		S	S			8
J12			S	S	S	S		S	S	S	S	S		S	S	S	S	13
J13	S		S	S	S	S		S	S	S	S	S	S	S	S	S		14
J14	S	S	S	S	S	S		S	S	S		S	S	S	S	S	S	15
J15	S	S	S	S	S			S	S	S	S	S		S	S	S		13
J16	S			S	S			S		S		S			S			7
J17	S	S	S	S	S	S		S	S	S		S		S	S	S	S	14
HGC*	4	11	2	3	1	9	15	8	4	0	9	2	14	2	1	7	12	

*: "S" refers to susceptible reaction (disease scores of 3 and 4) while blank space refers to resistance reaction (disease scores of 0, 1 and 2).

Table 4. Reaction of 17 differential host cultivars to 17 Jordanian *R. secalis* isolates (on a scale of 0 to 4) and resulting pathotypes*.

Isolate	Armelle	Astrix	Athene	Igri	La-Mesita	Osiris	Pirate	Digger	Trebi	Kitchin	Steudelli	Bey	Atlas-46	Modoc	Forrajera	Abyssinian	Rihane-3	Pathotype
J111A	0	1	4	2	3	0	0	2	0	3	0	0	0	1	3	0	1	J01
J121A	0	0	3	4	4	0	1	1	2	4	0	3	0	4	3	2	1	J02
J31B	4	4	4	4	4	4	4	2	4	4	4	4	2	4	4	4	2	J03
J51B	4	2	4	3	3	1	2	3	3	3	3	3	2	4	3	4	3	J04
J21C	3	0	0	2	1	0	0	2	2	3	0	2	2	3	2	0	0	J05
J31C	3	2	3	4	3	3	1	2	4	4	0	3	2	4	3	3	0	J06
J21D	4	2	4	4	4	3	2	3	4	4	3	4	2	3	4	3	1	J07
J61D	4	4	4	3	4	3	2	1	4	4	3	4	2	4	4	3	1	J08
J121D	3	0	3	2	4	0	1	0	3	4	2	3	2	4	4	2	0	J09
J11E	4	4	4	4	4	2	3	3	4	4	4	4	3	4	4	0	3	J10
J21F	2	0	4	4	4	0	0	2	3	3	0	3	2	3	3	2	0	J11
J51F	1	2	4	3	3	3	2	3	3	4	4	3	1	4	4	4	3	J12
J101F	3	1	4	3	3	3	0	3	4	3	3	4	3	3	4	3	1	J13
J31G	4	3	4	3	4	4	0	3	4	4	0	4	3	4	4	3	3	J14
J121G	4	3	4	4	3	2	0	4	4	4	3	3	1	4	4	3	1	J15
J71H	3	0	2	3	3	0	0	3	2	4	0	4	2	0	4	2	2	J16
J91H	4	4	4	4	4	3	0	4	4	4	2	4	2	4	4	4	3	J17

*: Disease score on a 5 point scale (0 to4) where 0 = no visible symptoms; 1 = small lesions confined to leaf tips and brown to gray necrotic spots; 2 = somewhat larger lesions; 3 = larger and coalescing lesions with distinct margins and 4 = total collapse of the leaf with no discrete lesions within the wilted area.

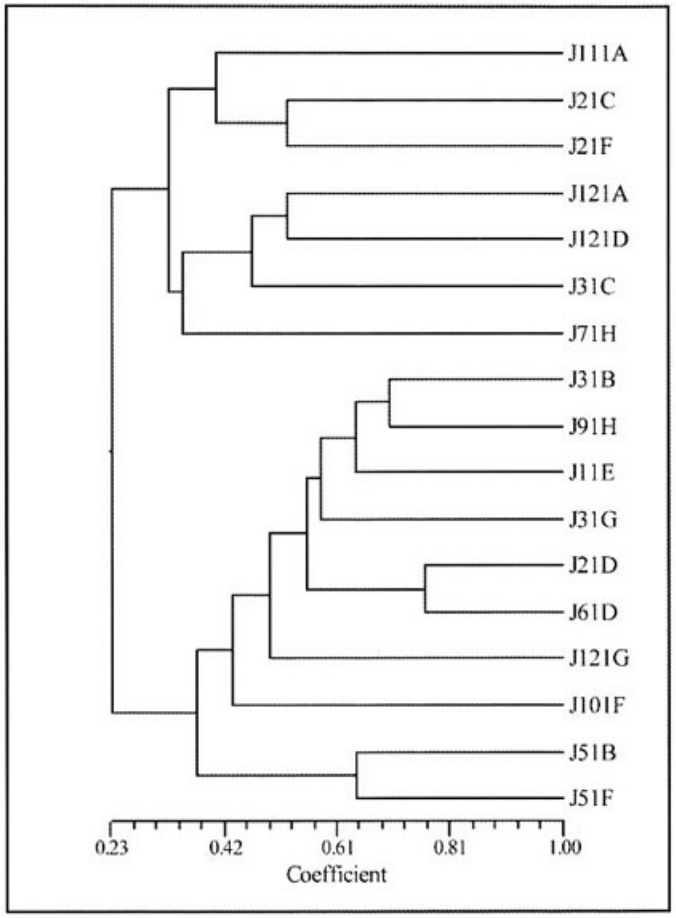

Figure 2. Phenogram of *Rhynchosporium secalis* isolates based on virulence data, using the 0 to 4 disease assessment scale. Jaccard's coefficient in the Numerical Taxonomy and Multivariate Analysis System for personal computers (NTSYS-pc; Exeter Software) generated distance matrix data of the virulence data. The name of the isolates is coded: J stands for country of origin (Jordan), followed by isolate reference number in each sampling stations and letters 'A' to 'H' refering to individual sampling stations in the field.

Discussion

The amount of genetic variation maintained within a population indicates how rapidly a pathogen population could adapt to resistant hosts and fungicides (24). Our study suggests high genetic diversity as shown by high genotype diversity which was 95% of the theoretical maximum with 35 unique MLHTs among 36 isolates, and by the moderately high gene diversity (0.39). Our finding is in agreement with those of McDonald *et al.* (27) who found 60 *R. secalis* genotypes among 61 isolates sampled from a single field in Australia.

The observed relatively large G_{ST} value in the current study suggest that the population comprises a mosaic of independent genotypes without significant migration between different locations in the field and the observed distribution of genetic variation is in agreement with the hypothesis that a genetically diverse founding population, possibly and as yet an undescribed teleomorph of *R. secalis*, provided the initial inoculum in the field, but was followed by rapid evolution of new variation in the absence of sexual recombination, probably resulting from high rate of mutation within few cycles of fungal infection as has been observed by Williams *et al.* (45). The significantly high linkage disequilibrium observed in this population gives supporting evidence that pathogen possess mechanisms of reproduction other than sexual recombination. We found similarly significant levels of linkage disequilibrium in a number of Jordanian *R. secalis* populations as measured using SSR markers (unpublished data).

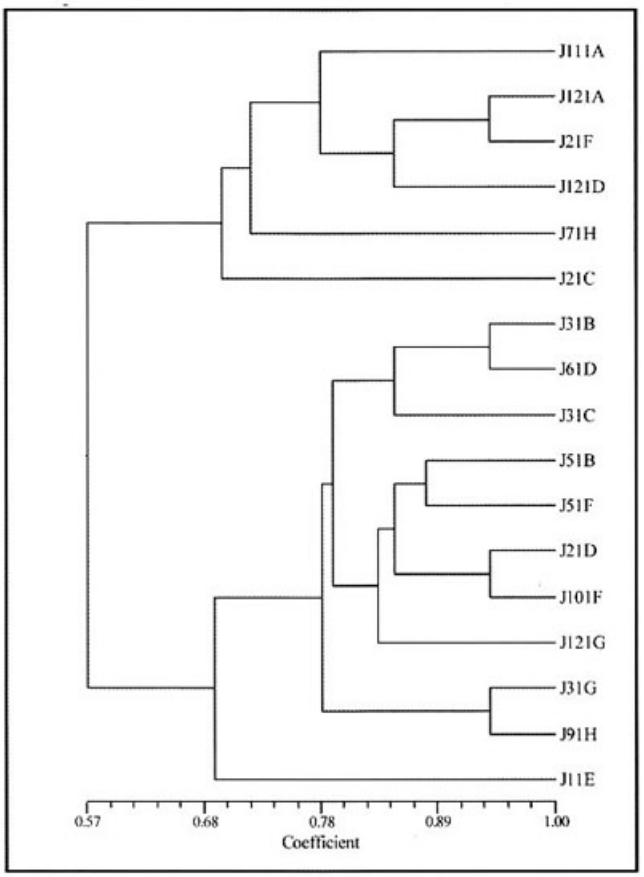

Figure 3. Phenogram of *Rhynchosporium secalis* isolates based on virulence data, using host reaction category, as R (resistant) and S (susceptible). Jaccard's coefficient in the Numerical Taxonomy and Multivariate Analysis System for personal computers (NTSYS-pc; Exeter Software) generated distance matrix data of the virulence data. The name of the isolates is coded: J stands for country of origin (Jordan), followed by isolate reference number in each sampling stations and letters 'A' to 'H' refering to individual sampling stations in the field.

The fact that the majority of the genetic diversity (57%) was distributed within the $1m^2$ sampling area in the field is in agreement with previous findings in which the majority of total RFLP diversity in Australian populations (27,38) was distributed within the smallest spatial scale of 1 m^2 sampling area. The fact that each of the Jordanian isolates, that was tested for virulence on the set of differential cultivars, was virulent on at least three of the differential cultivars may indicate that the pathogen population in Jordan possesses high diversity in virulence and that the pathotypes possess

unnecessary virulence, more virulence than is needed to overcome the resistance genes carried by the currently cultivated barley cultivars in Jordan. Similar unnecessarily high virulence in the pathogen had been described in other regions (1, 35, 37, 48). The virulence spectrum observed in the present study seems to be broader than most previously reported investigations (Table 5) though comparisons among results of such studies may be difficult for the fact that different sets and number of differentials were used and even we have noticed that the same differentials from different seed sources failed to show a similar reaction to the same pathotype. Nevertheless, the fact that isolates differed in their virulence despite being collected from within a single field or from different plants within an area of 1 m^2 is consistent with previous findings where spores from different lesions collected at the same location and even different spores from the same lesion were classified into different pathotypes (7, 35).

Table 5. Number of pathotypes/races of *R. secalis* identified in different countries

Country	Number of Isolates	Number of Races/ Pathotypes	Number of Differential cultivars	Ref.
Australia	203	35	21	3
California, USA	175	75	14	17
New Zealand	149	4	18	10
Ontario (Canada)	352	20	5	48
Japan	38	36	14	11
Denmark	38	28	23	20
Alberta (Canada)	265	52	12	47
Australia (East)	276	20	15	7
Australia	319	5	15	6
California	723	362	14	49
Italy	100	17	13	9
Norway	42	32	22	37
Canada	111	45	20	41

Schürch *et al.* (39) characterized few Jordanian isolates of *R. secalis* using four differential cultivars and reported that none of the isolates were virulent on 'Atlas' and 'Atlas-46' though they were virulent on 'Ingrid' and 'Turk NIL'. Cultivars 'Ingrid', 'Atlas' and 'Turk NIL' were not included in the current study, however, 'Atlas-46' had shown resistance reaction against 14 of the 17 Jordanian isolates. Observed discrepancy between our result and those of Schürch *et al.* (39) could mainly probably be due to the use of different seed sources in the virulence tests.

Test for association between the AFLP marker data and virulence characters of selected isolates showed no significant correlation between virulence pattern and AFLP marker data. The absence of association between virulence and molecular attributes is in agreement with previous reports where Newton *et al.* (32) found no clear relationship between molecular and pathogenicity attributes and Goodwin *et al.* (12) reported similar lack of association between pathogenicity and isozyme phenotype for *R. secalis* isolates occurring in Idaho and Oregon.

Influence of selection pressure resulting from commercial cultivars would be unlikely to explain the unusually high virulence diversity observed in the Jordanian population of *R. secalis* since local cultivars are mainly grown in the country. However, wild *Hordeum* species could serve as alternate hosts of *R. secalis* in Jordan since they are found widely distributed in the country and might possibly serve as sources of pathogenically diverse and complex pathotypes of the pathogen as was believed to be the case in Australia. In Australia, diverse sources of more complex pathotypes in the grass species have been indicated to influence the pathogenic variation of *R. secalis* populations (1). However, further studies should be conducted to determine whether *R. secalis* populations from the wild relatives of the host are influencing the genetic structure of the pathogen population on cultivated barley and whether there exists significant gene exchange among the populations on wild relatives and cultivated barley.

The current study has shown that the pathogen population in Jordan is highly diverse both in virulence and genetically. None of the resistance genes present in the differential host cultivars included in the current study seem to offer effective resistance against the pathotypes identified. Such high level of genetic and virulence diversity may indicate that the Jordanian *R. secalis* population is structured in such a way that major gene resistance, including pyramided genes, could be rendered ineffective as to provide long lasting protection. Pathogens with a mixed reproductive system are those considered as the highest risk pathogens. Though the sexual stage of *R. secalis* has not been described yet, it is believed that *R. secalis* could possibly have a mixed reproductive system and there exists a high degree of gene/genotype flow among populations of the pathogen (25). Hence for such pathogens breeding efforts should concentrate on quantitative resistance that will need to be renewed regularly to stay ahead of the pathogen.

الملخص

كيروس مالوس، أ.، عمور يحياوي، أحمد أبو بلان، سريبادا أودوبا ومايكل بوم. 2005. الاختلافات الوراثية وتباين شراسة مجتمع الفطر *Rhynchosporium secalis* مسبب السفعة الحقلية على الشعير ضمن حقول الأردن. مجلة وقاية النبات العربية، 23: 133-140.

تم تحديد البنية الوراثية لمجتمع فطر *R. secalis* المعزول بشكل هرمي من حقول الأردن (الرابا) باستخدام تقانة AFLP. لقد تم تعريف 34 نمط متعدد المواقع من مجموع 35 عزلة بغالبية تنوع ننمطها الوراثي 95% من الحد الاعظمي المفترض. توزعت التباينات الوراثية الرئيسة (57%) ضمن متر مربع واحد كمساحة تجريبية في الحقل. وقد أشارت التباينات الوراثية العالية والارتباط غير المتوازن المعنوي إلى إمكانية امتلاك الفطر لآليات تباين متجدد في غياب تكاثره الجنسي. اختبرت مجموعة من 17 عزلة لتحديد شراستها على مجموعة من 17 صنف وقسمت إلى 17 نمط إمراضي مختلف. لقد أبدت الأنماط الإمراضية درجات من الشراسة لثلاثة أصناف تفريقية على الأقل، ولم يظهر أي صنف تفريقي مقاومة لجميع الأنماط الإمراضية. تراوحت درجات تعقد النمط الإمراضي ما بين 3 إلى 15 بمتوسط 11. ولم يكن هناك علاقة واضحة أو ارتباط معنوي ما بين نتائج AFLP وشراسة العزلات المختبرة. أشارت نتائج هذه الدراسة إلى أن مجتمع هذا الممرض في الأردن يمتلك تنوعا وراثيا عاليا وشراسة غير ضرورية (unnecessary virulence) وبالتالي فإن استخدام المورث الرئيس المقاوم قد لا يؤمن حماية طويلة الأمد تحت ظروف الأردن.

كلمات مفتاحية: البنية الوراثية، AFLP، السفعة الحقلية، الأردن.

عنوان المراسلة: عمور يحياوي، المركز الدولي للبحوث الزراعية في المناطق الجافة (ايكاردا)، حلب، سورية؛ البريد الالكتروني: a.yahyaoui@cgiar.org

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