RESEARCH NOTE

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Genetic diversity and recombination within populations of *Fusarium pseudograminearum* from western Canada

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Summary. Genetic diversity within populations of *Fusarium pseudograminearum* isolated from wheat grains from the Canadian provinces of Alberta and Saskatchewan was investigated. Three restriction enzymes (*Eco*RI, *Hae*III, and *Pst*I) were used to carry out restriction analysis of the nuclear ribosomal DNA (nrDNA) intergenic spacer region (IGS region) and eight primers were used to generate inter-simple sequence-repeat (ISSR) molecular markers. Our study indicated substantially high genetic diversity within these two populations, but low genetic differentiation and frequent gene flow among populations. The IGS data showed no genetic distinction between the two Alberta populations and only minor genetic differentiation between the Saskatchewan and Alberta populations. Analysis of molecular variance indicated that most genetic variability resulted from differences among isolates within populations. Multilocus linkage disequilibrium analysis suggested a panmictic population genetic structure and the occurrence of significant recombination in *F. pseudograminearum*. Regular gene flow and random mating between isolates from different populations could result in novel genotypes with both improved pathological and biological traits. [**Int Microbiol** 2006; 9(1):65-68]

Key words: *Fusarium pseudograminearum* · *Gibberella coronicola* · genetic diversity · gene flow · genetic recombination

Introduction

Fusarium pseudograminearum Aoki and O'Donnell (teleomorph: *Gibberella coronicola* Aoki and O'Donnell), formerly known as *F. graminearum* group 1, is a haploid, heterothallic fungal pathogen belonging to *Fusarium* section *Discolor* [2]. Over the past few years, *F. pseudograminearum* has emerged as a major causal agent of crown-rot disease in cereal crops in various parts of the world [5]. The infection of cereal crops with *F. pseudograminearum* can result in a reduction in both grain yield and grain quality due to contamination with mycotoxins [4]. This species of *Fusarium* was identified in Canada only recently, and it has generally been isolated from the cereal-growing areas of western Canada. Isozyme analysis of *F. pseudograminearum* showed identical fingerprints among isolates [8], whereas phylogenetic analysis, using DNA sequence data from coding regions, demonstrated two genetic groups among the four isolates examined [15]. Although these studies provided useful preliminary information regarding the genetic structure of this fungus, there is still a need for further investigations to elucidate the genetic diversity and evolution of *F. pseudograminearum*.

The aim of the present study was to examine the genetic diversity within populations of *F. pseudograminearum* collected from infected wheat seed grown in the western Canadian provinces of Alberta and Saskatchewan. Restriction analysis of the nuclear ribosomal DNA (nrDNA) intergenic spacer region (IGS) and inter-simple sequence-repeat (ISSR) molecular markers were employed to analyze the genetic diversity and evolutionary dynamics of *F. pseudograminearum*. Both of these molecular markers have been used

extensively to study the population genetics of various eukaryotes and have provided important genetic information relevant to the resolution of various genetic and evolutionary properties in a range of fungi [7,12,18,19].

Materials and methods

Fungal isolates and DNA extraction. Twenty-six isolates of *F. pseudograminearum* were isolated from infected wheat grains collected from the western Canadian provinces of Alberta and Saskatchewan (Table 1). The single-spored isolates were grown on potato-dextrose agar (PDA) plates at room temperature for 7 days. The tissue was harvested from the isolates grown on PDA, ground in liquid nitrogen, and DNA was extracted using the procedure described previously [9]. The identity of the isolates was established using a PCR-based approach that had been developed for *F. pseudograminearum* [2].

DNA fingerprinting and gel electrophoresis. The nrDNA IGS region was amplified from all isolates using primers, reagents, and conditions as described previously [11]. From each isolate, 10 µl of the amplified IGS product was digested with three different restriction enzymes (*Eco*RI, *Hae*III, and *Pst*I) in separate reactions carried out according to the manufacturer's recommendations (Invitrogen, Canada). The digests were size-fractionated through a 2% agarose gel containing ethidium bromide, visualized, and photographed under ultraviolet light using a Gel Documentation System (Kodak, Canada). The resulting IGS fingerprints were examined to identify the number of haplotypes among the tested isolates; based on these results, seven isolates representing different IGS haplotypes were selected for ISSR analysis (Table 1). ISSR fingerprinting was carried out using the procedures and the eight ISSR primers described in our previous study [13]. The ISSR analysis was repeated and bands that appeared in both amplifications were regarded as markers in this study. The resulting fingerprints from both (IGS and ISSR) analyses were scored as presence (1) or absence (0) of the bands for each isolate.

Analysis of the molecular data. Genetic diversity within a population was calculated from the binary data using the average gene diversity over loci [13]; the genotypic diversity, using the equation described previously [21]; and the level of polymorphism. Fixation index (F_{ST}), and gene flow (Nm) estimates [16,20] were used to infer genetic differentiation between populations. Analysis of molecular variance (AMOVA) was applied to partition the genetic diversity into, among, and within population components [6]. All statistical measures were computed using the software Arlequin, version 2.0, a software for population genetic data analysis [University of Geneva, Switzerland]. Recombination in *F. pseudograminearum* was estimated using the rd statistic as installed in the software MultiLocus [1]. The rd statistic is based on the variance of the genetic distance between pairs of isolates, compared to the corresponding variance in null data created through randomization of observed data under the expectation of random mating. In the present study, molecular marker data were randomized 1000 times to create a null distribution against which the observed rd value was compared. The observed rd statistic for a recombining organism is expected to be non-significant from the rd statistic inferred from the null data [1].

Results and Discussion

Restriction analysis of the IGS region resulted in 18 markers, of which 12 were polymorphic, whereas ISSR analysis resulted in 120 markers, of which 118 were polymorphic characters. Restriction analysis of the PCR-amplified IGS products resulted in five unique haplotypes among 26 isolates examined (Fig. 1, Table 1). The genotypic diversity calculated from the IGS data was relatively high in all populations, with the highest (0.980) found in the Alberta 2000 populations, followed by the Saskatchewan 2000 and Alberta 1998 populations, with genotypic diversities of 0.727 and 0.683, respectively. The genotypic diversity calculated from the ISSR data was 1.0, as all the isolates examined revealed different banding patterns. The average gene diversity over loci, inferred from the IGS data, was lower in population samples from Alberta (mean ± standard deviation: Alberta 1998, 0.079 ± 0.153; Alberta 2000, 0.054 ± 0.158) than in those from Saskatchewan (0.213 ± 0.173), whereas the ISSR results indicated similar values for samples from the two provinces (Alberta 1998, 0.394 ± 0.297; Saskatchewan 2000, 0.370 ± 0.245). However, there were no significant differences in the average gene diversity over loci among populations for both of the datasets, as was evident from the large standard deviations. The AMOVA results demonstrated that most of the genetic variation (94.17%) in the IGS region of *F. pseudograminearum* was due to within-population variability, while only 5.83% of the total variation occurred among populations. The within- and among-population variabilities for the ISSR data were 88.42% and 11.58%, respectively. The genetic diversity and the partitioning of genetic variation in *F. pseudograminearum* populations observed in our study are in agreement with the estimates of genetic diversity reported by other researchers for closely related *Fusarium* species, such as *F. graminearum* [10,13,21]. There are substantial similarities in the pathological, physiological, and ecological properties of the different species of *Fusarium* causing diseases in cereals [3]. Considering previous observations as well as the results of the present study, it seems that these *Fusarium* species also follow similar genetic and evolutionary trends.

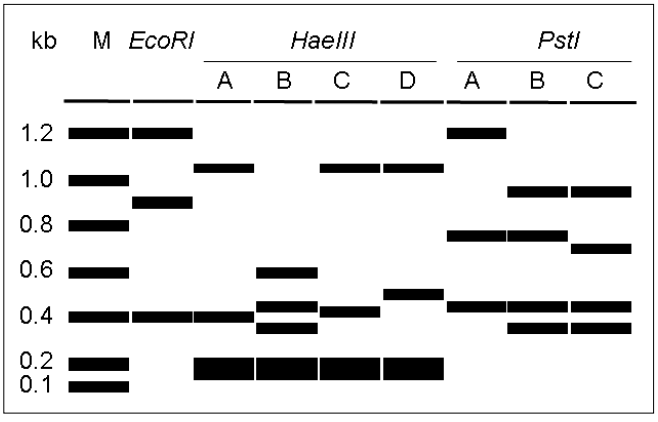

Fig. 1. Restriction patterns of the intergenic space region (IGS) of *Fusarium pseudograminearum.* M, size standard marker, in kilobases (kb).

Table 1. Isolates of *Fusarium pseudograminearum* examined in this study

	Origin				Restriction pattern		
Isolate	Province*	Crop district	Location	Year	*Eco*RI	*Hae*III	*Pst*I
A2-98-11	AB	2	Carseland	1998	A	A	A
A2-98-13	AB	2	Drumheller	1998	A	C	A
A2-98-14	AB	2	Drumheller	1998	A	A	A
A2-98-15	AB	2	Drumheller	1998	A	A	A
A2-98-18	AB	2	Nobleford	1998	A	C	A
A2-98-30	AB	2	Tempest	1998	A	C	B
A2-98-31	AB	2	Turin	1998	A	A	A
A4-98-01	AB	4	Ervick	1998	A	A	A
A4-98-02	AB	4	Forestburg	1998	A	A	A
A5-98-02	AB	5	Alix	1998	A	C	A
A5-98-03	AB	5	Ellerslie	1998	A	A	A
A2-00-11	AB	2	Vulcan	2000	A	C	A
A2-00-02	AB	2	Carseland	2000	A	A	A
A3-00-01	AB	3	Beiseker	2000	A	A	A
A4-00-01	AB	4	Legacy	2000	A	A	A
A4-00-02	AB	4	Provost	2000	A	A	A
A4-00-04	AB	4	Viking	2000	A	C	A
A4-00-05	AB	4	Viking	2000	A	C	A
S1B-00-1	SK	IB	Kipling	2000	A	D	C
S2A-00-2	SK	2A	Truax	2000	A	C	A
S2A-00-3	SK	2A	Truax	2000	A	D	C
S3AN-00-1	SK	3AN	Gravelbourg	2000	A	C	A
S3AS-00-1	SK	3AS	Limerick	2000	A	C	A
S9B-00-1	SK	9B	Lloydminster	2000	A	C	A
S9B-00-2	SK	9B	Lloydminster	2000	A	A	A
S9B-00-3	SK	9B	Lloydminster	2000	A	B	A

*AB, Alberta. SK, Saskatchewan.

The estimates of F_{ST} and Nm, determined from the IGS data, demonstrated low genetic differentiation and high gene flow among populations. The IGS data showed no genetic distinction between the two Alberta populations (F_{ST} = 0, Nm = infinite), while only minor genetic differentiation was found between the Saskatchewan and Alberta populations. The F_{ST} value between the Alberta 1998 and Saskatchewan 2000 populations was 0.114, whereas it was 0.088 between the Alberta 2000 and Saskatchewan 2000 populations. The corresponding gene flows (Nm) between these populations were 4.38 and 5.68, respectively. ISSR markers generated from the Alberta 1998 and Saskatchewan 2000 populations also resulted in genetic differentiation and gene flow values (F_{ST} = 0.130, Nm = 3.18) similar to those obtained with the IGS data. These results are consistent with a panmictic population genetic structure and further suggest that frequent gene flow and random mating between populations of *F. pseudograminearum* are predominant evolutionary forces determining the evolution and development of this fungus in western Canada. This explanation is additionally supported by results of the measure of multilocus linkage disequilibrium (rd statistic), which revealed the occurrence of significant recombination in *F. pseudograminearum* populations. The observed rd value, determined from the IGS data, was 0.2558, which was not significantly different (p = 0.254) from the rd value expected under random mating (rd = 0.2376). Similarly, for the ISSR data, the observed rd value (rd = 0.0031) and the rd value expected under random mating (rd = 0.0029) were also not significantly different (p = 0.427). The occurrence of significant recombination is in agreement with the dynamics of sexual development in the natural populations of *F. pseudograminearum* [17].

Overall, our study revealed a substantially high genetic diversity within populations of *F. pseudograminearum* recovered from infected wheat seeds in the provinces of Alberta and Saskatchewan in western Canada. Low genetic differentiation and high gene flow were found among the population samples examined, and significant recombination within populations. These results suggest that regular gene flow and random mating between isolates from different populations could result in new and novel genotypes with both improved pathological and biological traits. As this species has so far been identified in a limited area of the western prairies of Canada, its potential evolution into new, more virulent genotypes is of concern. In fact, both the area affected and the severity of diseases attributable to this pathogen might increase. Thus, there is a need for ongoing monitoring of the incidence and distribution of *F. pseudograminearum* in Canada. In addition, formulation

of an integrated strategy for the management of diseases caused by this fungus deserves attention.

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Diversidad genética y recombinación en poblaciones de *Fusarium pseudograminearum* del oeste de Canadá

Resumen. Se investigó la diversidad genética en poblaciones de *Fusarium pseudograminearum* aisladas de semillas de trigo de las provincias candienses de Alberta y Saskatchewan. Se usaron tres enzimas de restricción (*Eco*RI, *Hae*III, and *Pst*I) para analizar los marcadores moleculares de las regiones espaciadoras intergénicas (IGS) del DNA ribosómico del núcleo (nrDNA) y de los fragmentos generados entre repeticiones de secuencias sencillas (fragmentos ISSR). Nuestro estudió reveló una gran diversidad genética en ambas poblaciones, pero poca diferenciación genética y un flujo genético frecuente entre las poblaciones. Los datos relativos a las IGS no mostraron diferencias genéticas entre las dos poblaciones de Alberta estudiadas y sólo una ligera diferenciación entre las poblaciones de Alberta y de Saskatchewan. El análisis de la varianza molecular indicó que la variabilidad genética respondía en su mayor parte a diferencias entre aislamientos dentro de las poblaciones. El análisis del desequilibrio en el ligamiento genético sugería una estructura genética de la población de tipo panmíctico y la existencia de una recombinación significativa en *F. pseudograminearum*. Un flujo genético regular y el apareamiento al azar de aislamientos de poblaciones distintas podría producir nuevos genotipos con características biológicas mejores o patológicas. [**Int Microbiol** 2006; 9(1):65-68]

Palabras clave: *Fusarium pseudograminearum* · *Gibberella coronicola* · diversidad genética · flujo genético · recombinación genética

Diversidade genética e recombinação em populações de *Fusarium pseudograminearum* do oeste do Canadá

Resumo. Foi averiguada a diversidade genética em populações de *Fusarium pseudograminearum* isoladas de sementes de trigo das províncias candenses de Alberta e Saskatchewan. Foram utilizadas três enzimas de restrição (*Eco*RI, *Hae*III, and *Pst*I) para analisar os marcadores moleculares das regiões espaçadoras intergênicas (IGS) do DNA ribossômico nuclear (nrDNA) e dos fragmentos gerados entre repetições de seqüências simples (fragmentos ISSR). Nosso estudou revelou uma grande diversidade genética em ambas populações, mas pouca diferenciação genética e um fluxo genético freqüente entre as populações. Os dados relativos às IGS no mostraram diferenças genéticas entre as duas populações de Alberta estudadas e apenas uma leve diferenciação entre as populações de Alberta e de Saskatchewan. A análise da variânza molecular indicou que a variabilidade genética correspondia em sua maioria a diferenças entre isolados de uma mesma população. A análise do desequilíbrio de ligação gênica sugeriu uma estrutura genética da população de tipo panmítico e a existência de uma recombinação significativa em *F. pseudograminearum*. Um fluxo gênico regular e o apareamiento ao acaso de isolamentos de populações diferentes poderia produzir novos genotipos com características biológicas melhores ou patológicas. [**Int Microbiol** 2006; 9(1):65-68]

Palavras chave: *Fusarium pseudograminearum* · *Gibberella coronicola* · diversidade genética · fluxo genético · recombinação genética