Genetic Diversity of *Gibberella zeae* Isolates from Manitoba

W. G. D. Fernando, J. X. Zhang, M. Dusabenyagasani, X. W. Guo, and **H. Ahmed,** University of Manitoba, Department of Plant Science, Winnipeg, MB R3T 2N2, Canada; and **B. McCallum,** Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB R3T 2M9, Canada

ABSTRACT

Fernando, W. G. D., Zhang, J. X., Dusabenyagasani, M., Guo, X. W., Ahmed, H., and McCallum, B. 2006. Genetic diversity of *Gibberella zeae* isolates from Manitoba. Plant Dis. 90:1337-1342.

Gibberella zeae (anamorph *Fusarium graminearum*) causes Fusarium head blight, one of the most important diseases of cereals in the Canadian prairies for the last decade. In 2002, 60 isolates of *G. zeae* were collected and single spored from naturally infected spikes of wheat from Carman and Winnipeg in Manitoba. These isolates were compared using vegetative compatibility analysis and polymerase chain reaction (PCR)-based sequence related amplified polymorphisms (SRAP). Sixteen vegetative compatibility groups (VCG) were found among the 50 isolates tested. Five VCGs were found in the two locations, five in Carman and six in Winnipeg. Eight SRAP primer pairs amplified 90 polymorphic DNA fragments from 60 isolates and identified 59 distinct haplotypes. Among seven pairs of isolates, each pair from a distinct spike, four had isolates with different VCGs and six comprised different SRAP haplotypes. Principal component analysis and UPGMA separated the dataset into two main groups, each with isolates from both locations. The analysis of molecular variance also revealed that 75 and 20% of the variance was associated with differences among individual isolates and varieties sampled, respectively. Geographic location was not a significant source of variation at $P = 0.05$ and accounted for only 4% of total variance. A low correlation between VCG and SRAP marker data was detected. This study showed that, although genetic diversity is high among *G. zeae* isolates, Carman and Winnipeg collections have a similar genetic makeup and are likely part of the same population. The significant proportion of variance accounted by the variety compared with the geographic origin of isolates suggests that seedborne inoculum might have contributed to the genetic diversity within the *G. zeae* collection under study.

Gibberella zeae (Schwein.) Petch (anamorph *Fusarium graminearum* Schwabe) is a plant pathogen of various plant species including barley, wheat, maize, rice, carnation, and other small cereals with a worldwide distribution (28). The taxonomy of *G. zeae* has been updated since 2000. Previously recognized as a panmictic species spanning continents, *G. zeae* is now considered to be a clade comprising nine phylogenetically distinct species defined using genealogical concordance phylogenetic species recognition (29,33). The status of *G. zeae* in Canada and Manitoba according to the new taxonomy is yet to be established. *G. zeae* causes Fusarium head blight (scab), a disease that has become economically devastating on barley and wheat in North America for the last two decades because of successive epidemics (14,22,32). Infection of cereals by *Fusarium* species reduces grain yield and quality, especially through grain contamination by mycotoxins that are harmful to humans and livestock (34). Fusarium head blight is weather dependent, precipitation being the main factor (15,22). No single control measure is effective against Fusarium head blight. However, intensive research aimed to develop resistant cultivars and biological control tools is going on because integrated management helps reduce losses (9,13).

Information on fungal population variabilities is important in order to better understand the source and development of disease outbreaks and to help develop and plan deployment of control measures. Several methods have been used to characterize *G. zeae* isolates. A nonexhaustive list includes morphological traits, vegetative compatibility analysis (VCA), mycotoxin production, and molecular methods (12).

VCA, which uses vegetative incompatibility, is the prevention of somatic fusion between individuals that occurs frequently and has been extensively investigated in ascomycetes (6,16,20,25). In many species studied, including *G. zeae*, a large number of vegetative compatibility groups (VCG) have been found (2,12,36). Molecular markers are also useful tools in the analysis of genetic variation in populations of plant-pathogenic fungi. A number of molecular techniques are available for studying the genetic relationships within and among fungal populations within a species. Protein based methods such as isozymes, and DNA-based methods including restriction fragment length polymorphisms (RFLP) and polymerase chain reaction (PCR) have been applied to population analyses of plant pathogens such as *F. graminearum* (7,11,36). Although each method has both strengths and limitations, the amplified fragment length polymorphism (AFLP) was until recently considered the most effective because it is highly reproducible and polymorphic compared with other methods such as RFLP, randomly amplified polymorphic DNA (RAPD), and simple sequence repeats (SSRs) (30). Sequence related amplified polymorphism (SRAP), a relatively new method based on two-primer PCR amplification, produces reproducible and polymorphic markers as AFLP with added advantages of simplicity and low cost because there are fewer steps involved (17,35). It has been used in gene tagging of *Brassica* species (17) as well as population analysis of *Apiosporina morbosa,* the fungal pathogen of black knot of *Prunus* spp. (10,37).

The objective of this work was to investigate the impact of geographic location and host variety on the genetic diversity of *G. zeae* isolates from several wheat varieties grown in two 80-km-distant locations of the province of Manitoba, Canada.

MATERIALS AND METHODS

Fungal isolates and vegetative compatibility analysis. Sixty isolates of *G. zeae* were collected from spikes of wheat varieties showing scab symptoms at two University of Manitoba agriculture field research stations located in Carman and Winnipeg (Table 1). Six varieties were sampled in Carman and 10 in Winnipeg (Table 1). Twenty-two isolates within the collection were from 11 different spikes, each spike with two isolates from different spikelets on the head. Spikelets with Fusarium head blight symptoms were used for isolation according to the Nash and Snyder method (24). Fungal cultures were further single-spored, maintained on synthetic nutrient agar (SNA) medium, and identified using keys for *Fusarium* spp. (4,26,27). Fifty isolates, including seven pairs from different spikes, were also

Corresponding author: W. G. D. Fernando
E-mail: D_Fernando@Umanitoba.ca

Current address of J. X. Zhang: USDA-ARS, Department of Plant Sciences, University of Arizona, Tucson 85721.

Current address of H. Ahmed: Alberta Research Council, Vegreville, AB T9C 1T4, Canada.

Accepted for publication 1 June 2006.

DOI: 10.1094/PD-90-1337

tested with VCA by pair-wise isolate confrontation in petri dishes with potato dextrose agar (PDA; Difco Laboratories, MD, USA) medium as previously described (21).

DNA extraction. Mycelia of each isolate were prepared in a flask with 75 ml of potato dextrose broth (PDB; Difco). To obtain mycelia, PDB flasks were inoculated with a 0.5-ml suspension of approximately 10^6 conidial spores per milliliter of an isolate. The flasks were incubated at room temperature for 7 days without agitation. A mycelial pad was formed and harvested by vacuum filtration through two layers of sterilized Miracloth (Calbiochem, CN Biosciences, Inc., La Jolla, CA). Mycelia were rinsed twice with sterilized distilled water and stored at –80°C until lyophilized. DNA of each isolate was extracted according to Lodhi et al. (19) without polyvinylpyrrolidone. All DNA extracts were quantified using a spectrophotometer and adjusted to a final concentration of 5 ng μl^{-1} for PCR analysis.

Amplification of SRAPs. The SRAP technique was used as a tool to analyze genetic diversity within the *G. zeae* collection. Thirty-six pairs of SRAP primers provided by G. Li (Department of Plant Science, University of Manitoba, Canada) were screened for the PCR amplification, and the following eight primer pairs, which provided polymorphic and reproducible markers (Table 2), were selected for generating the SRAP data: ODD9/ODD15, ODD9/EM2, ODD14/ODD32, ODD15/ODD30, ODD30/DC1, ODD15/EM1, ODD30/EM1, and DC1/RP1. PCR amplification reaction was performed in a 15-µl reaction volume containing 15 ng of template DNA, 0.4 µM each of two primers, 0.75 units of *Taq* polymerase (Fisher brand), 100 mM Tris-HCl (pH 8.0), 500 mM KCl, 1.5 mM $MgCl_2$, and 0.1 mM each of dNTPs. The fragments were amplified in a programmable thermal controller (Genius, TECHNE Ltd., Cambridge, UK). The first five cycles were run at 94°C for 1 min, 35°C for 50 s, and 72°C for 1 min, for denaturing, annealing, and extension, respectively. Then the remainder of the amplification was 36 cycles at 94°C for 50 s, 50°C for 50 s, and 72°C for 1 min. The amplified PCR products were separated by electrophoresis, using a denaturing 5% polyacrylamide gel containing 7.5 M urea. Gels were then silver stained with the Promega kit (Promega, Madison, WI, USA) according to the manufacturer's specifications. PCR replicates using the same set of primers and isolates and different DNA preparations of the same isolates were conducted with all combinations of isolates and primers to check the repeatability of results. The presence and absence of all fragments between molecular sizes of 50 and 500 bp were scored for each isolate. Bands representing molecular sizes larger than 500 bp or less than 50 bp were not scored because the resolution was insufficient to discriminate between bands of various molecular sizes.

Statistical analysis. Populations were defined according to their geographic origin. Amplified fragments were manually recorded as present (1) and absent (0). Several methods were used to analyze SRAP data. Principal component analysis (PCA) using covariance matrices was performed with the Program SAS (SAS Institute, Cary, NC, USA) to determine main components that could define significant structures within the dataset. The software Population Genetics Analysis (POPGENE, version 1.32; Molecular Biology and Biotechnology Center, University of Alberta, Edmonton, Canada) was used for estimation of standard population genetics parameters. The analysis of molecular variance (AMOVA; 8) included in Arlequin program (31) was performed and allowed to partition the total genetic variance within and among the populations. To investigate the relationship between VCGs and SRAPs, a simple Mantel test was performed with VCG and SRAP information from 50 isolates using the Zt program (1).

RESULTS

VCA showed that mycelia from compatible isolates overgrew and mixed with each other but incompatible isolates produced a typical barrier at the confrontation zone (Fig. 1). Sixteen VCGs were found among the 50 isolates tested. VCGs 1, 3, 6, 7, and 16 were only present in Carman, VCGs 9, 10, 11, 13, 14, and 15 were only in Winnipeg, and VCGs 2, 4, 5, 8, and 12 were in both locations (Table 1). We have also identified different VCGs from individual spikes: four out of seven pairs of

Table 2. Sequences of the sequence related amplified polymorphism (SRAP) primers used to amplify *Gibberella zeae* genomic DNA

Primer name	Primer sequence
DC1	5′-TAA ACA ATG GCT ACT CAA G-3′
ODD30	5′-GCG ATC ACA GAA GGA AGG T-3′
ODD14	5′-TCG GTC TTT GTC GTT TCT A-3′
ODD32	5′-ACT GTG ATG TCG TTA CTG AT-3′
ODD9	5′-AGT TCC TCA GAC GCT ACC-3′
ODD15	5′-GCG AGG ATG CTA CTG GTT-3′
EM1	5′-GAC TGC GTA CGA ATT AAT-3′
EM2	5′-GAC TGC GTA CGA ATT TGC-3′
RP1	5′-CAT TGT GGA TGG CAT CTG A-3′

Table 1. Characteristics of *Gibberella zeae* isolates collected from wheat in Carman and Winnipeg, Manitoba, in 2002

Location	Variety	Isolate[a]	VCG[b]
Carman	Readymade	F14, F23a, F24a	NA, NA, NA[c]
Winnipeg	Readymade	F130, F131, F135	10, 11, NA
Carman	Osprey	F47, F51b, F52b, F53	1, 1, 1, 2
Carman	CDC Raptor	F54c, F55c, F56d, F57d, F58, F60	3, 4, 3, 5, 6, 6
Carman	CDC Raptor	F62e, F63e, 64f, 65f, F66g, F68g, F69h, F70h	6, 6, 6, 2, 7, NA, 8, 7
Winnipeg	CDC Raptor	F157, F159, F160	5, 14, 8
Carman	UM5089	F88, F89i, F90i	7, 7, 7
Winnipeg	UM5089	F166, F169j, F170j, 174	15, NA, 4, 4
Carman	UM5116	F92	12
Winnipeg	UM5116	F179, F180, F181, F183, F185, F186	5, 4, 10, 9, 15, 10
Winnipeg	Norster	F115	NA
Winnipeg	Ketrel	F119	9
Winnipeg	Norwin	F123, F128	11, 2
Winnipeg	Clair	F137	4
Winnipeg	Falcon	F144k, F145k, F146, F148	4, 4, 12, 13
Carman	CDC Teal	F191, F194, F212, F213, F217, F218, F222, F223	NA, 2, 2, 2, 16, 16, NA, NA
Winnipeg	CDC Teal	F229, F231	4, 8

[a] Isolates followed by the same letter were from the same spike.
[b] Vegetative compatibility group.
[c] Not analyzed.

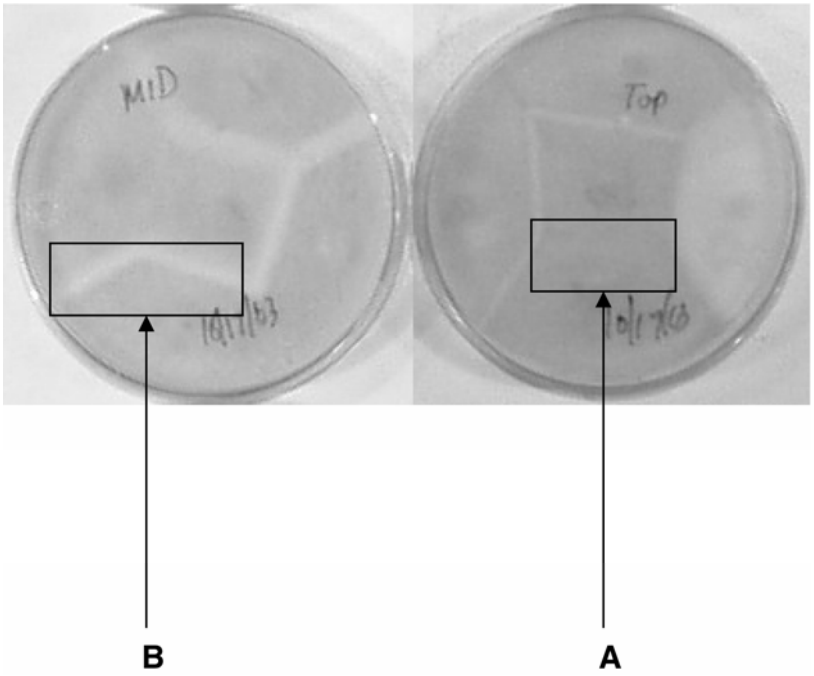

Fig. 1. Vegetative compatibility analysis. A = compatible, and B = incompatible between nonmutant *Gibberella zeae* isolates on potato dextrose agar (PDA) medium.

isolates, each pair from a distinct spike, comprised isolates with different VCGs (Table 1). Ninety polymorphic SRAP fragments were present in 60 *G. zeae* isolates using eight pairs of the SRAP primers (Fig. 2). Only two isolates (F51 and F52) out of 60 had the same SRAP haplotype and likely represent the same colonization event. Statistical analysis of SRAP data revealed that even though most isolates were different, the composition of Carman and Winnipeg subcollections was comparable. The percentage of polymorphic loci is identical and the Shannon indices suggest that genotypes might be similarly distributed in both locations (Table 3).

PCA revealed that the first two principal components accounted for 31.72% of the total variance and the remaining ones explained less than 10% each (PCA_1 = 20.11, PCA_2 = 11.28, PCA_3 = 6.29, etc.). According to the plot involving the first two main components, SRAP markers with positive contribution to the first principal components characterize isolates from variety Osprey, but SRAP markers with negative contribution characterize isolates from variety Readymade (Fig. 3). The SRAP

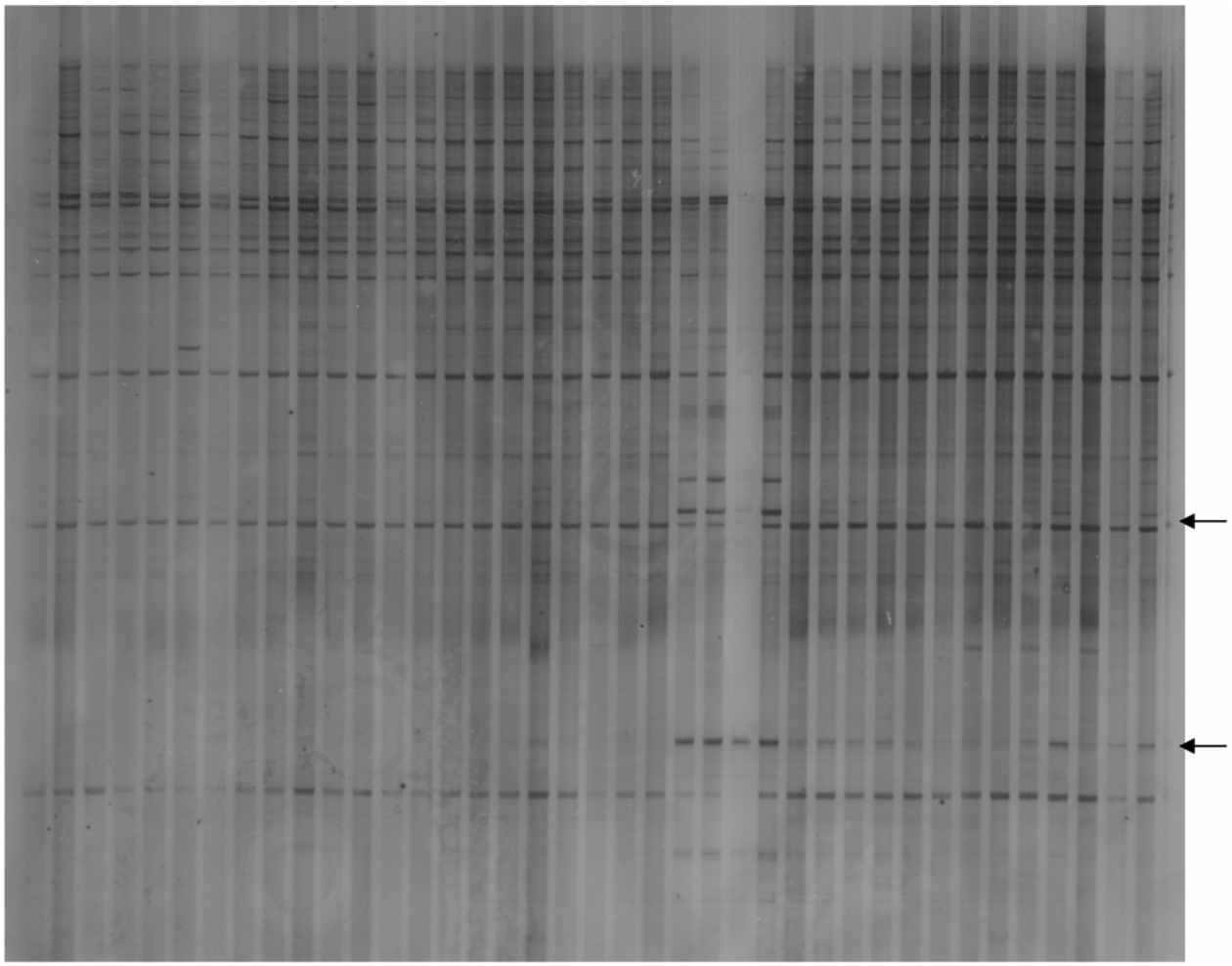

Fig. 2. A polyacrylamide gel that separated sequence related amplified polymorphism (SRAP) amplification products from *Gibberella zeae* isolates using the ODD9 and EM2 primers. Arrows show polymorphic SRAP markers. From left to right, lanes represent polymerase chain reaction (PCR) products from isolates F57, F58, F60, F62, F63, F64, F65, F66, F68, F69, F70, F88, F89, F90, F92, F115, F119, F123, F128, F130, F131, F135, F137, F144, F145, F146, F148, F157, F159, F160, F166, F169, F170, F174, F179, F180, F181, F183, and F185.

dataset revealed that at least five markers are associated to PCA_1. Three of them are present in isolates from variety Readymade but absent in isolates from variety Osprey. The other two markers are absent in isolates from variety Readymade but present in isolates from variety Osprey. The second principal component shows that most isolates from variety CDC Teal are characterized by SRAP markers with positive contribution, but those with negative contribution characterized most isolates from variety CDC Raptor (Fig. 3). According to the SRAP marker dataset, at least three markers are associated to PCA_2. Two of them are present in all but one isolate from variety CDC Teal but absent in all isolates from variety CDC Raptor. The third marker is present in all isolates from variety CDC Teal but absent in all but one isolate from variety CDC Raptor.

The AMOVA revealed that 75% of the total variance was due to differences among individual isolates and 20% of the variance was associated to the varieties from which isolates were collected within both locations. Geographic location contribution was not significant at $P = 0.05$ and accounted for only 4% of the total variance (Table 4). Such observation was not only supported by diversity indicators (Table 3), which revealed a great deal of migration (Nm = 8.4), close Shannon indices, and identical percentage of polymorphic loci, but was also confirmed by values of genetic identity and distance between isolates from Carman and Winnipeg of 0.98 and 0.02, respectively, that suggested nearly identical population composition. The correlation between VCGs and SRAP, although significant, is small ($r = 0.1067$; $P = 0.0015$) and does not allow association of the two characteristics.

DISCUSSION

This study found a high number of VCGs, although they were less important than previously reported for *G. zeae* (2,3,13,34). Contrary to the previous findings for *G. zeae* populations from Kansas and North Dakota (36), our results revealed that isolates within the same VCG were not clones because most of them had different SRAP haplotypes. The difference between these results may be explained by the different discriminatory power provided by the VCA methods used. Our PDA-based VCA method may be less discriminatory than the nitrate nonutilizing mutant based method used in the previous study (36). This study may have underestimated the number of VCGs by identifying only macroscopic incompatibility phenotypes that involve several vegetative incompatibility (*vic*) genes, in contrast to the nitrate mutant method that can detect differences associated to one *vic* gene. Hypoviruses have been reported in *G. zeae* and could also explain the difference in the results (5). Analyses of their transmission in *Cryphonectria parasitica* has revealed that *vic* genes appear to be additive in

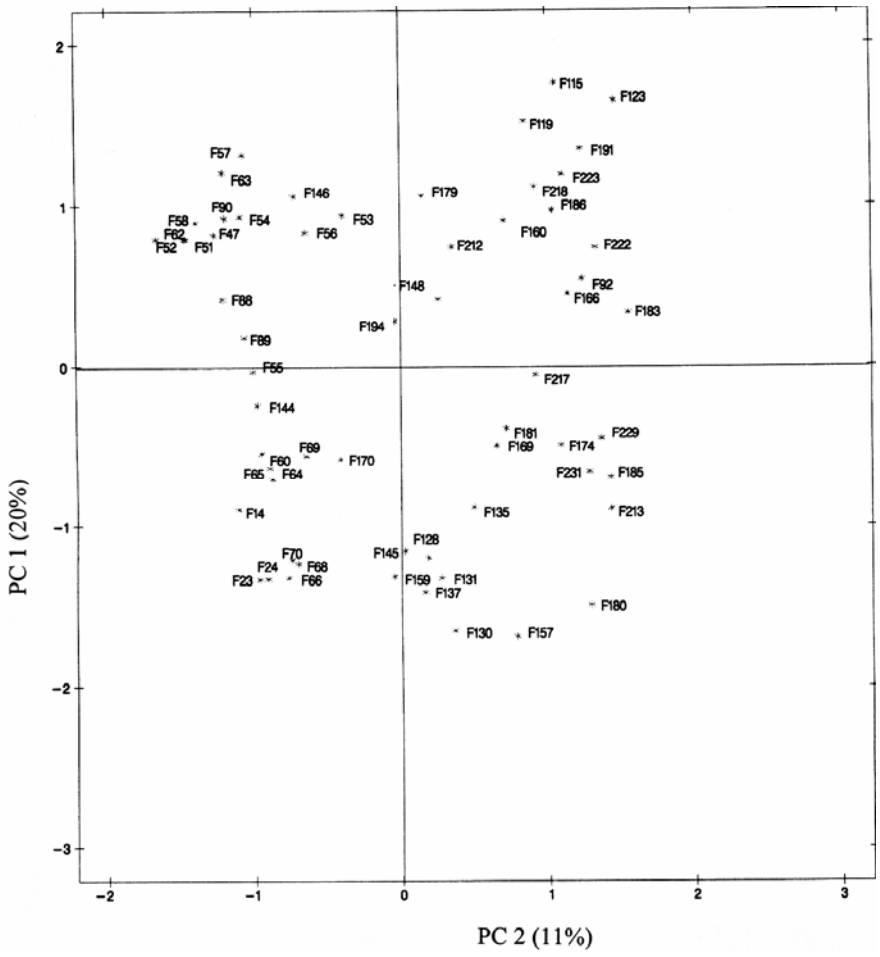

Fig. 3. Principal component analysis score plot of 60 *Gibberella zeae* isolates based on 90 sequence related amplified polymorphism (SRAP) markers. PC1 = first principal component; PC2 = second principal component. The first two principal components account for 31% of the total variance associated to the 90 SRAP markers.

Table 4. Analysis of molecular variance (AMOVA) for 90 sequence related amplified polymorphism (SRAP) markers assayed in 60 *Gibberella zeae* isolates from Carman and Winnipeg, Manitoba, in 2002

Source	df	Variance	% Variance	*P*
Among locations	1	0.31949	3.91	0.0574
Among varieties within locations	14	1.69678	20.78	<0.0001
Within varieties	44	6.14813	75.30	<0.0001

Table 3. Genetic diversity indicators of the *Gibberella zeae* populations from Carman and Winnipeg, Manitoba

Population	Population size	g[a]	s[b]	p[c]	H[d]	Gst[e]	Nm[f]
Carman	33	32	0.249	63.3	0.156	–	8.431
Winnipeg	27	27	0.270	63.3	0.173	0.056	–

[a] Number of genotypes in population.
[b] Shannon index.
[c] Percentage of polymorphic loci (99% criterion).
[d] Average unbiased proportion heterozygosity.
[e] Gst = coefficient of gene variation.
[f] Nm = number of migrants.

some vegetative incompatible reactions (18).

The identification of different VCGs from individual spikes suggested that at least two different infection events and sources had occurred. We cannot, however, establish the extent of wheat spike multiple infection by *G. zeae* because only two isolates per head were collected from a limited number of spikes. However, our results are consistent with previously reported data from barley and wheat (20,36).

SRAP results are in agreement with findings about the high diversity of *G. zeae* isolates from the United States and eastern Canada based on RAPD, ISSR, and AFLP markers (7,23,36). Such diversity is indicative of the frequency of sexual recombination undergone by *G. zeae* in three Canadian prairie provinces: Alberta, Manitoba, and Saskatchewan (23). According to PCA, each of the principal components accounted for a proportion of the total variance of 20% or less, which suggests that, despite the high diversity, no well-defined groups could be characterized within the used fungal collection, and therefore the isolates likely represented quite a homogeneous gene pool.

According to the AMOVA, the variety that was the source of isolates accounted for more than five times the variance explained by geographic location. Geographic location was not a significant source of variation at $P = 0.05\%$. However, the variance associated to the variety could be a little overestimated because of the limited number of isolates from each variety. Our experimental design could not allow differentiation of the contributions of the variety per se and the seedborne inoculum, but we assume from the results that at least both together might have had some significant impact on the diversity of *G. zeae* populations. The seedborne inoculum hypothesis might also explain the high level of identity and the number of migrants estimated between Carman and Winnipeg, because the wheat seeds used in the two locations were from the same lots. The movement of cereal seeds within the Canadian prairies have been considered the main cause of lack of geographical structures within *G. zeae* (23). Thus, making representative *G. zeae* collections may require sampling several wheat varieties. The weak correlation between VCG and SRAP data is related to the scale of genome sampling provided by these methods, SRAPs being randomly distributed markers, in contrast to VCGs, which are based on only several *vic* genes. The relative importance of variety over geographic location identified by this study suggests that inoculum migration together with sexual recombination are probably the main factors affecting the genetic diversity of *G. zeae* populations in Carman and Winnipeg. Appropriate design could be used with the SRAP markers developed by this study to estimate the impact of different varieties on *G. zeae* genetic diversity regardless of seed sources. There is an active research program at the Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba, in the University of Manitoba campus where resistance to Fusarium head blight is under development using marker-assisted selection. Some relatively resistant cultivars such as AC Barrie and AC Cadillac are already in farmers' fields as part of an integrated management approach in the Canadian prairies. These SRAP markers could therefore help to assess whether different management schedules (i.e., different combinations of crop rotation, tillage, and wheat cultivars) affect the genetic diversity of *G. zeae* populations.

ACKNOWLEDGMENTS

This study was financially supported by Natural Science and Engineering Research Council of Canada (NSERC) and Agriculture Research and Development Initiative (ARDI) of Manitoba. We thank Yu Chen and Lorne Adam for technical assistance with the formatting of figures.

LITERATURE CITED

1. Bonnet, E., and Van de Peer, Y. 2002. Zt: A software tool for simple and partial Mantel tests. J. Stat. Software 7:1-12.
2. Bowden, R. L., and Leslie, J. F. 1992. Nitrate-nonutilizing mutants of *Gibberella zeae* (*Fusarium graminearum*) and their use in determining vegetative compatibility. Exp. Mycol. 16:308-315.
3. Bowden, R. L., and Leslie, J. F. 1994. Diversity of *Gibberella zeae* at small spatial scales. (Abstr.) Phytopathology 84:1140.
4. Burgess, L. W., Liddell, C. M., and Summerell, B. A. 1988. Laboratory Manual for Fusarium Research, 2nd ed. University of Sydney, Sydney, Australia.
5. Chu, Y.-M., Jeon, J.-J., Yea, S.-J., Kim, Y.-H., Yun, S.-H., Lee, Y.-W., and Kim, K.-H. 2002. Double stranded RNA mycovirus from *Fusarium graminearum*. Appl. Environ. Microbiol. 68:2529-2534.
6. Cortesi, P., and Milgroom, G. M. 1998. Genetics of vegetative incompatibility in *Cryphonectria parasitica*. Appl. Environ. Microbiol. 64:2988-2994.
7. Dusabenyagasani, M., Dostaler, D., and Hamelin, R. C. 1999. Genetic diversity among *Fusarium graminearum* strains from Ontario and Quebec. Can. J. Plant Pathol. 21:308-314.
8. Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479-491.
9. Fernando, W. G. D., Miller, J. D., Seaman, W. L., Seifert, K., and Paulitz, T. C. 2000. Daily and seasonal dynamics of airborne spores of *Fusarium graminearum* and other *Fusarium* species sampled over wheat plots. Can. J. Bot. 78:497-505.
10. Fernando, W. G. D., Zhang, J. X., Chen, C. Q., Remphrey, W. R., and Schurko, A. 2005. Molecular and morphological characteristics of *Apiosporina morbosa* (Schw.) Arx., causal agent of black knot in *Prunus* spp. Can. J. Plant Pathol. 27:364-375.
11. Gale, L. R., Chen, L.-F., Hernick, C. A., Takamura, K., and Kistler, H. C. 2002. Population analysis of *Fusarium graminearum* from wheat fields in Eastern China. Phytopathology 92:1315-1322.
12. Gilbert, J., Abramson, D., McCallum, B., and Clear, R. 2001. Comparison of Canadian *Fusarium graminearum* isolates for aggressiveness, vegetative compatibility, and production of ergosterol and mycotoxins. Mycopathologia 153:209-215.
13. Gilbert, J., and Fernando, W. G. D. 2004. Epidemiology and biological control of *Gibberella* zeae/*Fusarium graminearum*. Can. J. Plant Pathol. 26:464-472.
14. Gilbert, J., and Tekauz, A. 2000. Review: Recent developments in research on fusarium head blight of wheat in Canada. Can. J. Plant Pathol. 22:1-8.
15. Hooker, D. C., Schaafsma, A. W., and Tamburic-Ilincic, L. 2002. Using weather variables pre- and post-heading to predict deoxynivalenol content in winter wheat. Plant Dis. 86:611-619.
16. Leslie, J. F. 1993. Fungal vegetative compatibility. Annu. Rev. Phytopathol. 31:127-150.
17. Li, G., and Quiros, C. F. 2001. Sequence-related amplified polymorphism (SRAP), a new system based on a simple PCR reaction: Its application to mapping and gene tagging in *Brassica*. Theor. Appl. Genet. 103:455-461.
18. Liu, Y.-C., and Milgroom, M. G. 1996. Correlation between hypovirus transmission and the number of vegetative incompatibility (*vic*) genes different among isolates from a natural population of *Cryphonectria parasitica*. Phytopathology 86:79-86.
19. Lodhi, M. A., Ye, G. N., Weeden, N. F., and Reisch, B. I. 1994. A simple and efficient method for DNA extraction from grapevine cultures and *Vitis* species. Plant Mol. Biol. Rep. 12:6-13.
20. McCallum, B. D., Tekauz, A., and Gilbert, J. 2001. Vegetative compatibility among *Fusarium graminearum* (*Gibberella zeae*) isolates from barley spikes in southern Manitoba. Can. J. Plant Pathol. 23:83-87.
21. McCallum, B. D., Tekauz, A., and Gilbert, J. 2004. Barrage zone formation between vegetatively incompatible *Fusarium graminearum* (*Gibberella zeae*) isolates. Phytopathology 94:432-437.
22. McMullen, M., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.
23. Mishra, K. P., Tewari, J. P., Clear, R. M., and Turkington, T. K. 2004. Molecular genetic variation and geographical structuring in *Fusarium graminearum*. Ann. Appl. Biol. 145:299-307.
24. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
25. Nauta, M. J., and Hoekstra, R. F. 1996. Vegetative incompatibility in ascomycetes: Highly polymorphic but selectively neutral? J. Theor. Biol. 183:67-76.
26. Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* Species, An Illustrated Manual for Identification. Pennsylvania State University, University Park.
27. Niremberg, H. I. 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. Can. J. Bot. 59:1599-1609.
28. O'Donnell, K., Kistler, H. C., Tacke, B. K., and Casper, H. H. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Natl. Acad. Sci. USA 97:7905-7910.
29. O'Donnell, K., Ward, T. J., Geiser, D. M., Kistler, H. C., and Aoki, T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet. Biol. 41:600-623.
30. Powell, W., Morgante, M., Andre, C., Hanafey,

M., Vogel, J., Tingey, S., and Rafalski, A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Breed. 2:225-238.
31. Schneider, S., Roessli, D., and Excofier, L. 2000. Arlequin: A Software for Population Genetics Data Analysis. Ver 2.0. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva, Switzerland.
32. Sutton, J. C. 1982. Epidemiology of wheat blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Plant Pathol. 4:195-209.
33. Taylor, J. W., Jacobson, D. J., Kroke, S., Kasuga, T., Geiser, D. M., Hibbett, D. S., and Fisher, M. C. 2000. Phylogenetic species recognition and species concepts in Fungi. Fungal Genet. Biol. 31:21-31.
34. Ueno, Y., Nakajima, M., Sakai, K., Ishii, K., Sato, N., and Shimada, N. 1973. Comparative toxicology of trichothecene mycotoxins: Inhibition of protein synthesis in animal cells. J. Biochem. 74:283-296.
35. Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.
36. Zeller, K. A., Bowden, R. L., and Leslie, J. F. 2003. Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. Phytopathology 93:874-880.
37. Zhang, J. X., Fernando, W. G. D., and Remphrey, W. R. 2005. Genetic diversity and structure of the *Apiosporina morbosa* populations on *Prunus* spp. Phytopathology 95:859-866.