Virulence and molecular polymorphisms of the wheat leaf rust fungus *Puccinia triticina* in Canada from 1997 to 2007

Xiben Wang, Guus Bakkeren, and Brent McCallum

Abstract: Populations of *Puccinia triticina*, one of the casual agents of wheat leaf rust disease, in the pacific (British Columbia and Alberta), prairie (Manitoba and Saskatchewan), and eastern regions (Quebec and Ontario) of Canada from 1997 to 2007 were analyzed for virulence and genetic diversity by revealing expressed sequence tag derived simple sequence repeat (EST-SSR) polymorphisms. Since 1997, a significant shift in the virulence of *P. triticina* occurred across Canada. The diversity of *P. triticina* virulence phenotypes in Manitoba and Saskatchewan, as measured by Shannon and Simpson indexes, decreased due to the directional selection toward predominant virulence phenotypes, whereas it remained relatively constant in Quebec and Ontario. The clustering of *P. triticina* virulence phenotypes from 1997 to 2007 was similar to that found in previous years, and was correlated with virulence to leaf rust resistance genes *Lr2a*, *Lr2c*, and *Lr17a*. Distinct EST-SSR profiles were found in different groups of *P. triticina* virulence phenotypes based on virulence to *Lr2a*, *Lr2c*, and *Lr17a*. In addition, the population of *P. triticina* in Manitoba and Saskatchewan was different from that in Quebec and Ontario from 1997 to 2007, based on both virulence characteristics and EST-SSR genotypes.

Key words: Puccinia triticina, wheat leaf rust, virulence, simple sequence repeat and diversity.

Résumé : Les auteurs ont analysé les populations du *Puccinia triticina*, l'agent causal de la rouille foliaire du blé dans les régions du pacifique (Colombie britannique et Alberta), des prairies (Manitoba et Saskatchewan) et de l'est (Québec et Ontario) au Canada, de 1997 à 2007, en déterminant la virulence et la diversité génétique mises en évidence par les polymorphismes par le polymorphisme de marqueurs de type microsatellites dérivé d'étiquettes de séquences exprimées (EST-SSR). Depuis 1997, on observe un glissement significatif de la virulence du *P. triticina* à travers le Canada. La diversité des phénotypes de virulence du *P. triticina* au Manitoba et en Saskatchewan, telle que mesurée par les index de Shannon et de Simpson, adiminué dû à une sélection directionnelle vers des phénotypes virulents prédominants, alors que cette diversité est demeurée relativement constante au Québec et en Ontario. Le regroupement des phénotypes de virulence du *P. triticina* de 1997 à 2007, ne diffère pas de celui observé au cours des années antécédentes, et montre une corrélation avec la virulence des gènes de résistance à la rouille foliaire *Lr2a*, *Lr2c* et *Lr17a*. On observe des profils EST-SSR dans différents groupes de phénotypes de virulence du *P. triticina*, basés sur les gènes de virulence à *Lr2a*, *Lr2c* et *Lr17a*. De plus, la population du *P. triticina* du Manitoba et de la Saskatchewan s'éloigne de celle du Québec et de l'Ontario entre 1997 et 2007, en se basant à la fois sur les caractéristiques de virulence et sur les génotypes EST-SSR.

Mots-clés : Puccinia triticina, rouille foliaire du blé, virulence, séquence à simple répétition et diversité.

[Traduit par la Rédaction]

Introduction

Wheat leaf rust, caused by *Puccinia triticina* Eriks, is a serious production problem in many wheat growing areas worldwide, including Canada (Kolmer 2005; McCallum and Seto-Goh 2009). Depending on the level of resistance in wheat cultivars and the growth stage of crops at the time of initial infections, *P. triticina* can cause 5%–25% yield loss annually (Kolmer 1996; McCallum and Seto-Goh 2004). Although considerable resources have been devoted to breeding programs to develop wheat cultivars resistant to *P. triticina*, wheat cultivars with the long-lasting or durable resistance against this pathogen have been hard to obtain, owing to the fungus' genetic variability and capability of migrating over large geographical regions (Kolmer 2005; McCallum et al. 2007). Forty to 60 different virulence phenotypes of *P. triticina* can be found annually in North America (Kolmer 1999*b*; McCallum and Seto-Goh 2004, 2008). *Puccinia triticina* races virulent against newly deployed wheat leaf rust resistance genes can rapidly increase in frequency over large areas, which quickly render these resistance genes ineffective. In Canada, genes such as *Lr1*, *Lr13*, and *Lr14a* have been overcome, but others such as *Lr21*, *Lr22a*, and *Lr34* are still effective after a number of years in field grown cultivars (McCallum et al. 2007).

To monitor changes in virulence of *P. triticina* populations, field surveys have been conducted annually in North America since 1931. Because similar predominant virulence

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phenotypes were often found in the southern, central, and northern Great Plains of North America, it was proposed that populations of *P. triticina* in the Great Plains regions of the United States and the eastern prairie region of Canada constitute a single epidemiological unit. Epidemics are initiated from *P. triticina* urediniospores blown northwards into Canada from north-central states of the United States in spring and early summer (Kolmer 1989, 1999*b*). In contrast, many *P. triticina* virulence phenotypes found in the eastern region of Canada, including Quebec and Ontario, were different from those found in Manitoba and Saskatchewan (McCallum and Seto-Goh 2004, 2005). It is possible that *P. triticina* in Quebec and Ontario is capable of overwintering on susceptible winter wheats commonly grown in the region, which provides a local source of inoculum, in addition to inoculum blown in from the US (Kolmer 1999*b*). The combination of a susceptible host population and a local source of inoculum put the *P. triticina* population in Quebec and Ontario under less selection pressure to the wheat leaf rust resistance genes deployed. Quebec and Ontario could also receive inoculum from the eastern United States where different wheat cultivars are grown compared with the Great Plains (Kolmer et al. 2005; McCallum and Seto-Goh 2005). The number of samples collected in the pacific region, including Alberta and British Columbia, is limited (McCallum and Seto-Goh 2008), compared with the prairie and eastern regions of Canada, Among these samples, it appears that most virulence phenotypes found in Alberta and British Columbia also were present in Manitoba and Saskatchewan, suggesting that these two regions share a common source of inoculum. Nevertheless, some virulence phenotypes were unique to the pacific region (McCallum and Seto-Goh 2006, 2008).

In addition to differences in virulence characteristics, *P. triticina* populations from different regions of Canada were also different in their genetic background, based on random amplified polymorphic DNA (RAPD) (Kolmer et al. 1995) and amplified fragment length polymorphism (AFLP) analyses (Kolmer 2001*a*). For example, Kolmer (2001*a*) reported that *P. triticina* populations in Canada consisted of highly structured, distinct clusters and some isolates found in eastern Canada had virulence and genetic profiles that were distinct from those collected in western Canada.

From 1987 to 1997, virulence of *P. triticina* in Manitoba and Saskatchewan changed dramatically, owing to the introduction of new wheat leaf rust resistance genes in winter wheats grown in the Great Plains regions of the US. However, the phenotypic diversity of *P. triticina* in Manitoba and Saskatchewan remained fairly constant (Kolmer 1999*b*). In comparison, both virulence and phenotypic diversity of *P. triticina* in Quebec and Ontario was relatively constant. Since 1997, large shifts in virulence of *P. triticina* occurred in both the US (Kolmer et al. 2007) and Canada (McCallum and Seto-Goh 2006, 2008, 2009), where virulence patterns of *P. triticina* among different regions of Canada have changed dramatically (McCallum and Seto-Goh 2008).

The objectives of this study were to summarize results of wheat leaf rust surveys in Canada from 1997 to 2007, and to examine the relationship between genetic variability and virulence phenotypes in *P. triticina* in different regions of Canada using newly developed simple sequence repeat markers. Attributes of *P. triticina* populations in different regions of Canada including virulence and genetic diversity were compared. Such information on virulence and genetic diversity among *P. triticina* populations, combined with the knowledge of resistance genes deployed in Canadian wheat cultivars, will be highly valuable for the breeding of new wheat cultivars effective against this important fungal pathogen.

Material and methods

Determination of virulence phenotype for *Puccinia triticina* isolates used

Historical data of *P. triticina* virulence phenotypes in Canada were taken from annual wheat leaf rust surveys (Table 1), which were published in the *Canadian Journal of Plant Pathology* (McCallum and Seto-Goh 2002, 2004, 2005, 2006, 2008, 2009; Kolmer 1999*a*, 2001*b*). For the genetic and virulence analysis, a total of 69 common *P. triticina* virulence phenotypes found in Canada from 1997 to 2007 were used. For the purpose of comparison, eight virulence phenotypes found in Canada pre-1990s were also included. The infection types of *P. triticina* isolates were determined using a set of 16 standardized differentials [Set 1: *Lr1*, *Lr2a*, *Lr2c*, *Lr3*; Set 2: *Lr9*, *Lr16*, *Lr24*, *Lr26*; Set 3: *Lr3ka*, *Lr11*, *Lr17a*, *Lr30*; Set 4: *LrB*, *Lr10*, *Lr14a*, *Lr18*] according to the four letter code described by Long and Kolmer (1989).

Puccinia triticina EST-SSR genotypic analysis

For the extraction of total genomic DNA, fresh urediniospores were germinated on the surface of distilled water, and mats of germinated urediniospores were collected after 24 h. Spore mats were then vacuum-dried and ground with glass beads. Genomic DNA was extracted from spore mats using the method described by Kolmer (2001*a*). DNA concentration was determined by spectrophotometer readings at A_{260} and A_{280} and a working solution of 50 ng/μL was made. PCR was carried out in a 25 μL reaction volume containing 1× PCR buffer (Applied Biosystems, Streetsville, Ontario, Canada), 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 2.5 mmol/L each forward and reverse primers, 0.5 U *Taq* polymerase (Invitrogen, Burlington, Ont.) and 30 ng sample DNA. Amplifications were performed in a thermal cycler (GeneAmp™ PCR system 9700, Applied Biosystems) using the following temperature profile: initial denaturation step at 95 °C for 2 min, then 35 cycles at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min, followed by a final extension step at 72 °C for 5 min. PCR products were analyzed on a 6% polyacrylamide gel, visualized by silver staining (Chalhoub et al. 2007) and size in base pairs was calculated using a 25 bp DNA ladder (Invitrogen) as reference size standard. Alleles were scored based on length polymorphisms.

The M13-tailed primer method (Zhou et al. 2001) was used to label amplicons for the visualization of PCR products on a capillary DNA analyzer (Applied Biosystems). The forward primer was 5′-tailed with 23-base pair M13 (unit-43) sequence (AGGGTTTTCCCAGTCACGACGTTXXXXX) where X denoted the SSR-specific primer sequence. Twenty *P. triticina* EST-SSRs were used in this study (Table 2). PCR was carried out in a 12.5 μL reaction volume containing 1×

Table 1. Summary of *Puccinia triticina* virulence phenotypes used.

Isolate designation	Virulence code	*Lr 2a*	*Lr 2c*	*Lr 17a*	Group	Year
12-2	MDMJ	a*	a	a	I	1997
97	MBBJ	a	a	a	I	1997
63-3	KDGJ	v	v	v	V	1998
231-1	TDBG	v	v	a	V	2001
56-1	MCRJ	a	a	a	I	2001
163-1	PCMR	a	v	a	III	2001
120-1	MBTS	a	a	v	II	2002
219-3	KBGJ	v	v	a	V	2002
139-1	KBJJ	v	v	v	VI	2002
2	KBBJ	v	v	a	V	2002
10-3	MJBJ	a	a	a	I	2002
199-1	PBLR	a	v	a	III	2002
167-1	PCLR	a	v	a	III	2002
99-1	MBDS	a	a	v	II	2003
119-1	MBPS	a	a	v	II	2003
110-1	MCJJ	a	a	v	II	2003
14-1	TBDS	v	v	v	VI	2003
112-1	TJBJ	v	v	a	V	2003
109-1	SBDJ	v	v	v	VI	2003
229-1	TCGJ	v	v	a	V	2003
2-1	TBDJ	v	v	v	VI	2003
18-2	SDBJ	v	v	a	V	2003
231-3	MFGJ	a	a	a	I	2004
220-1	MCTS	a	a	v	II	2004
58-2	MCPS	a	a	v	II	2004
225-1	MBJS	a	a	v	II	2004
231-2	MCGJ	a	a	a	I	2004
15-1	TBBG	v	v	a	V	2004
214-2	PBDK	a	v	v	IV	2004
227-1	NBRT	a	v	a	III	2004
96	TBGJ	v	v	a	V	2004
42-1	TDBS	v	v	a	V	2004
216-3	PCRR	a	v	a	III	2004
115-1	MBDJ	a	a	v	II	2005
214-1	MBJD	a	a	v	II	2005
215-2	MCDS	a	a	v	II	2005
218-2	MBGJ	a	a	a	I	2005
228-1	NBBR	a	v	a	III	2005
215-1	MFDS	a	a	v	II	2005
170-2	MDBJ	a	a	a	I	2006
210-2	MCQK	a	a	a	I	2006
2-1	TNBG	v	v	a	V	2006
199-1	MDNS	a	a	v	V	2006
174-2	PBDG	a	v	v	IV	2006
169-2	MMDS	a	a	v	II	2006
74-2	TNPJ	v	v	v	VI	2006
13-2	TFBG	v	v	a	V	2006
237-1	MBPS	a	a	v	II	2007
48-2	TDBK	v	v	a	V	2007
153-2	TDBQ	v	v	a	V	2007
215-1	MBBK	a	a	a	I	2007
252-1	MGNS	a	a	v	II	2007
168-2	MDPN	a	a	v	II	2007
245-1	MFRS	a	a	a	I	2007
246-1	TFBS	v	v	a	V	2007
234-2	MLDK	a	a	v	II	2007
41-2	TDBT	v	v	a	V	2007

Table 1 (*concluded*).

Isolate designation	Virulence code	*Lr 2a*	*Lr 2c*	*Lr 17a*	Group	Year
248-2	TBBS	v	v	a	V	2007
251-1	MCNS	a	a	v	II	2007
2-2	MLDJ	a	a	v	II	2007
193-1	MLDS	a	a	v	II	2007
243-2	MLPN	a	a	v	II	2007
187-1	MDDS	a	a	v	II	2007
251-2	MFPS	a	a	v	II	2007
245-2	MFRJ	a	a	a	I	2007
172-2	TFBJ	v	v	a	V	2007
185-2	TDBJ	v	v	a	V	2007
30-1	TDGH	v	v	a	V	2007
188-2	MDPS	a	a	v	II	2007
136-1	FBMT	a	v	a	III	Pre-1997
214-1	MBPR	a	a	v	II	Pre-1997
49-2	MFMJ	a	a	a	I	Pre-1997
8	MFBJ	a	a	a	I	Pre-1997
62-2	TDRJ	v	v	a	V	Pre-1997
84-1	PBTR	a	v	v	IV	Pre-1997
151-2	KCGJ	v	v	a	V	Pre-1997
R9	SBDG	v	v	v	VI	Pre-1997

*"a" or "v" represents avirulent or virulent on corresponding wheat leaf rust differentials.

PCR buffer (Applied Biosystems), 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 1.25 μmol/L of each SSR specific primer, 0.5 μmol/L of 5′-fluorescently labelled M13 (unit-43) primer, 0.5 U *Taq* polymerase, and 30 ng sample DNA. M13 primers were tagged at the 5′-end with fluorescent labels HEX, 6-FAM, or NED to facilitate multiplexing. Amplifications were performed in a thermal cycler (GeneAmp PCR 9700) using the following temperature profile: initial denaturation step at 95 °C for 2 min, followed by 35 cycles at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min, followed by a final extension step at 72 °C for 5 min. Amplification products were analyzed using ABI3730 DNA analysis software Genographer (version 2). A fluorescent DNA size standard ranging from 50–500 bp was included in each run.

Data analysis

Virulence dissimilarity for each pair of isolates was calculated using the complement of the simple match coefficient, defined as $1–(d/n)$, in which d = number of matches between virulence of paired isolates; and n = total number of differential lines. A binary data matrix was also generated for all EST-SSRs based on the presence (=1) or absence (=0) of amplification products. The similarity of EST-SSR genotypes between each pair of isolates was determined using a Dice-coefficient: $2a/(a+b+c)$, in which a = bands present in isolate i and j; b = band present in isolate i, absent in isolate j; c = band present in isolate j, but absent in isolate I (Kolmer and Ordoñez 2007). The similarity matrix of EST-SSR genotypes was converted to a distance matrix by subtracting the Dice-coefficient from 1.

The dissimilarity matrices for both virulence and EST-SSRs were used to construct dendrograms using the SAHN clustering method in UPGMA (unweighted pair group arithmetic mean method) program in NTSYS-pc, version 2.1 (Exeter Software, Seatauket, New York). The 2-D principle coordinate analyses (2D-PCA) were performed on virulence and EST-SSR datasets using NTSYS-pc. To determine the correlation between the dendrogram and original similarity matrix, a co-phenetic value matrix of the dendrograms was generated using the COPH module in NYSYS-pc. The dissimilarity matrices derived from dendrograms based on virulence or EST-SSRs were transformed with the Dcenter module in NTSYS and degrees of the correlation between matrixes derived from virulence and EST-SSRs were determined with the MXCOMP module in NTSYS-pc. This multivariate approach was chosen to complement the cluster analysis with UPGMA and MODECLUS.

The dissimilarity matrices for virulence and EST-SSRs were both subjected to the non-parametric cluster analysis MODECLUS (1997, SAS Institute Inc., Cary, North Carolina) to determine the appropriate number of clusters within datasets. The rationale and advantages of MODECLUS over other clustering methods have been previously described (Harder et al. 2001). MODECLUS has no requirement for the statistical assumption of distribution of variables and resulting clusters are not biased toward having the same size. A smoothing parameter (K value) was used in MODECLUS to determine the number of clusters over different values. The K-value was plotted against the number of clusters and regions that were flat on the graph were regions of stability to be considered as candidates for appropriate K values for defining clusters. The clusters taken from the region of stability were then evaluated by subjecting the original data matrix to an ordination through canonical discriminant analysis (CDA) using the SAS CANDICS procedure (1997, SAS Institute Inc.).

Table 2. Repeat motif, primer sequence, and characteristics of *Puccinia triticina* EST derived simple sequence repeat markers.

Locus	Primer sequence[a]	Repeat motif	Ta (°C)	Na	Allele size range (bp)
83	F: ATGGATTTGGAGACCAGTCG R: GTTGAAAGATCTGGGGGTGA	(GA)n	60	2	292–298
6981	F: ACGTGGTGAGGTTTCTGCTC R: TTCCGTTTTTGAAAGCAAGC	(ATC)n	59	2	163–171
19	F: GTTCGGATACCCCGTTTCTC R: TTTGGAGCATGTTGTTTTGG	(ATC)n	60	2	148–153
5649	F: CAGACGACCATCAACATTCG R: CATGAACCAAACAAACAGCTTC	(CT)n	60	3	189–195
85	F: CCAAAATTATCCCGCCCTAT R: GCGAGGGGGTAGGAAGTAAT	(TTT)n	60	2	289–295
6259	F: GTTCAACACATTGCGCTGTT R: ATGGGTTGTGCAGATCGAGT	(TCA)n	59	2	238–244
2948	F: CACACACCACACAAAACCAA R: CCCAACAAGCTCGTGTCTTT	(GAT)	59	2	117–121
536	F: TGTTGCGAATTGATGGTACG R: GAAGTTCTGCTCTGCTGTCG	(AAA)n	60	2	180–186
3233	F: GTAAGCTCGCTTTGGCTACG R: TTTGGAGCATGTTGTTTCCA	(GA)n	60	2	165–167
5594	F: CGGACCAAACACAAAGGAAA R: CCCTGCGTTTAACACCTTGT	(GAT)n	60	4	205–217
189	F: TCTCAACCAAAAATCAATCTACG R: CTTCCACGAAGACGAAGCAC	(AT)	58	4	102–118
801	F: CAATGGTAGTGGCAAGCAAA R: GCACCTCTCACGCTCTTAGC	(TG)n	60	2	201–204
6863	F: TAGATGGGCACACAACCAAA R: AAGCAAAGTGCAAGGAGCAT	(CT)n	60	2	212–248
243	F: CTCACTCGCTCGCTTGTTCT R: GACGAAAAGATCGGGTTTGA	(CA)n+(AT)n	60	4	201–211
125	F: ATCGTGTCATGCAACCAAAA R: AGAGAGGGACGTGAGGGATA	(GAT)n	59	2	177–183
481	F: CCACAATCCTCCGTTCTGAT R: CGAAAGCAAAACACATGAGG	(TTT)n	60	3	192–199
639	F: TCTCCGCCTACCAACACTG R: AAAGGAGGGAGAGGGGAGG	(GAA)n	60	2	204–210
3145	F: TAGGTGCGTGGTTTTCATCA R: CAAATGAGAGCGACGAACAA	(TCTT)n	60	2	181–189
6542-2	F: TGTGATCTCGCCCGTACATA R: TGGGAATGATGGACACACAC	(CT)n	60	4	142–162
182-2	F: CGAATCCCTTGTCTTTTGCT R: TGTAGAGAGCGGGAGAAGAAA	(CT)n	59	2	172–174
6386	F: AATGAGGTGACTCGGATGGA R: GAAGAAGGCGAAGTTGTTGC	(CAT)n	59	2	193–199

[a]F, forward primer; R, reverse primer; Ta, annealing temperature; Na, number of alleles.

The diversity of virulence phenotypes of *P. triticina* in Canada was determined with Shannon's diversity index (*H*) and Simpson's Index of Diversity (1-D) as

$$H = -\sum\nolimits_{P_i} \log_e(P_i)$$

and

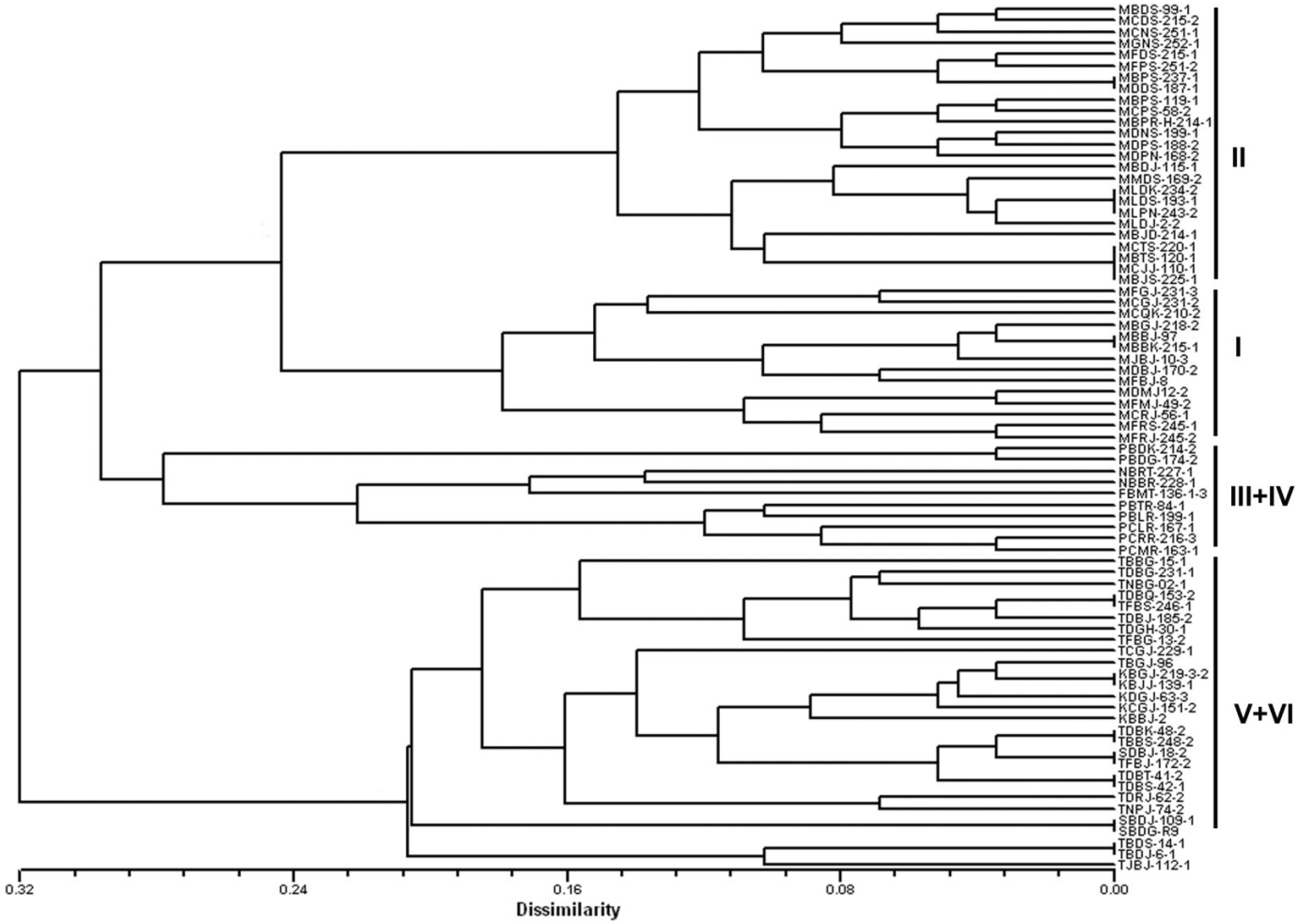
$$D = \sum n_i \frac{(n_i - 1)}{N(N - 1)}$$

where P_i is the frequency of the *i*th virulence phenotype, n_i is the number of *i*th virulence phenotype and N is total number of *P. triticina* isolates. Association between virulence to different resistance genes in *P. triticina* was measured using the *G* test of independence at the 5% confidence level (Sokal and Rohlf 1981).

Results

A total of 77 *P. triticina* virulence phenotypes were included in the cluster analysis (Table 1). The virulence dissimilarity matrix generated using the simple mismatch coefficient was analyzed with the UPGMA and MODE-

Fig. 1. Virulence dissimilarity dendrogram of *Puccinia triticina*. *Puccinia triticina* isolates representing 77 virulence phenotypes in Canada were analyzed based on unweighted pair group method with arithmetic means clustering, using the simple dismatch coefficient calculated based on virulence to 16 wheat leaf rust differentials. Six groups of *P. triticina* isolates were defined based on their virulence to *Lr2a*, *Lr2c*, and *Lr17a* including group I (avirulent on *Lr2a*, *Lr2c*, and *Lr17a*), group II (avirulent on *Lr2a* and *Lr2c*, virulent on *Lr17a*), group III (avirulent on *Lr2a*, virulent on *Lr2c*, and avirulent on *Lr17a*), group IV (avirulent on *Lr2a*, virulent on *Lr2c* and *Lr17a*), group V (virulent on *Lr2a* and *Lr2c*, avirulent on *Lr17a*), and group VI (virulent on *Lr2a*, *Lr2c*, and *Lr17a*).

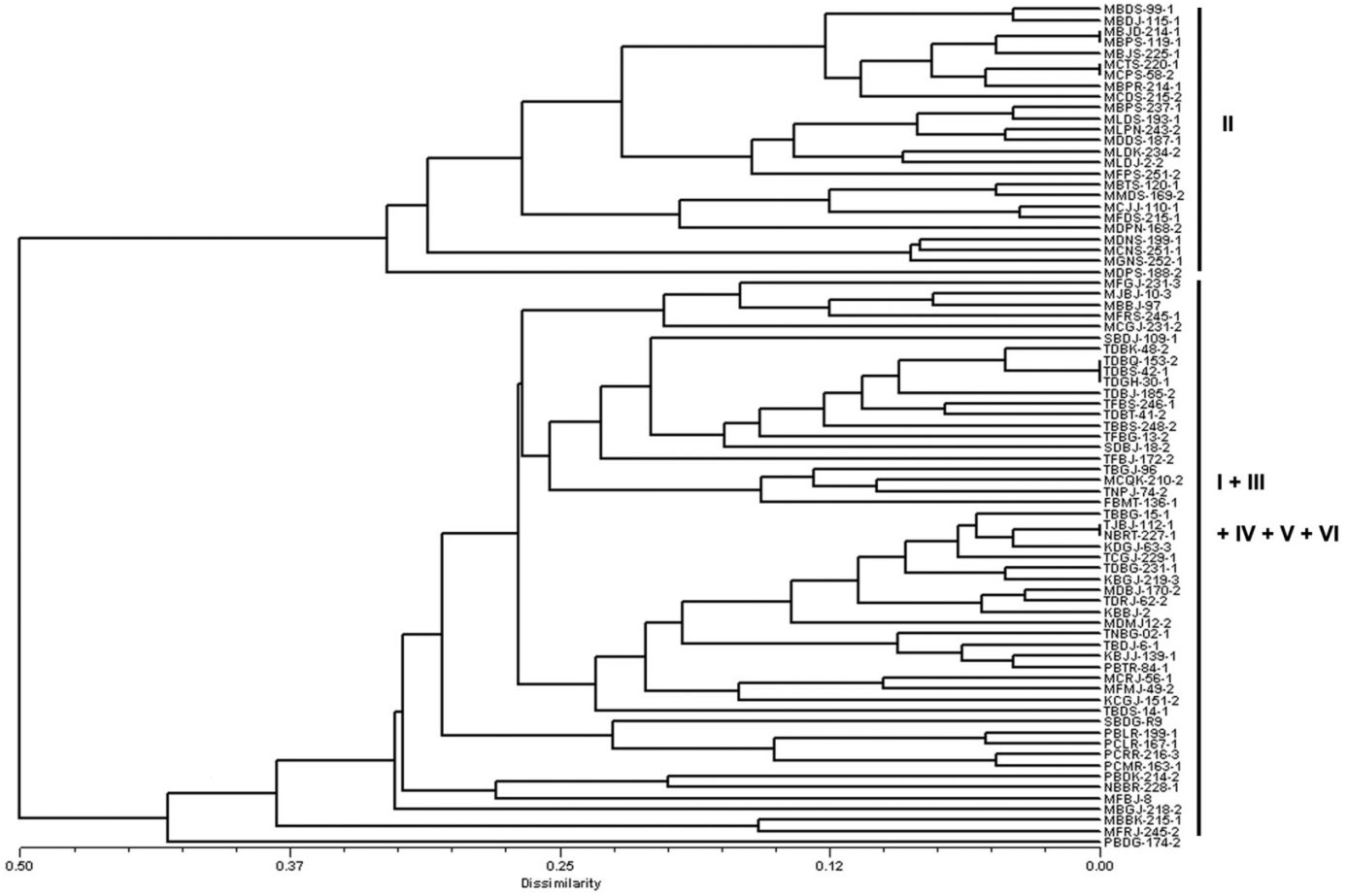


CLUS clustering methods. Based on the previous data of Wang et al. (2009) and Ordoñez and Kolmer (2009), *P. triticina* virulence phenotypes were divided into six groups based on virulence to leaf rust resistance genes *Lr2a*, *Lr2c*, and *Lr17a*. Group I was avirulent on *Lr2a*, *Lr2c*, and *Lr17a* and group II was avirulent on *Lr2a* and *Lr2c* but virulent on *Lr17a*. Group III contained isolates that were avirulent on *Lr2a*, virulent on *Lr2c*, and avirulent on *Lr17a*, whereas all isolates in group IV were avirulent on *Lr2a* but virulent on *Lr2c* and *Lr17a*. Group V and group VI were both virulent on *Lr2a* and *Lr2c*, but differed in virulence on *Lr17a* (Table 1, Figs. 1 and 2).

Three clusters of *P. triticina* virulence phenotypes were found when the virulence dissimilarity matrix was subjected to UPGMA analysis. Virulence phenotypes in groups I, II, III, and IV were separated from virulence phenotypes in group V and VI at 32% dissimilarity (Fig. 1). Within the first branch of the dendrogram, group III and IV virulence phenotypes were placed in one sub-branch which were separated from group II and I virulence phenotypes at 29% dissimilarity. The genetic dissimilarity matrix based on EST-SSRs was generated using the complement of dice coefficient and subjected to UGPMA and MODECLUS analysis. Two major clusters were separated at 51% dissimilarity when the EST-SSR dataset was analyzed with the UPGMA clustering method (Fig. 2). The first cluster constituted virulence phenotypes in group II and the second cluster contained the rest of the groups.

All virulence phenotypes were then visualized using a two-dimensional principal coordinate (2D-PCA) plot (Fig. 3A). The first two dimensions accounted for 73% of variations. All virulence phenotypes in group II were plotted in one region and separated from virulence phenotypes in other groups. Group I was situated between group II and V. With three exceptions, group III and IV virulence phenotypes were separated from those in group I, II, V, and VI. The distribution of virulence phenotypes in group V and IV was relatively dispersed in the graph, which showed overlap with virulence phenotypes in group I, III, and IV. The clustering structure within the virulence dissimilarity matrix was

Fig. 2. Genetic dissimilarity dendrogram of *Puccinia triticina* isolates. *Puccinia tritticina* isolates representing 77 virulence phenotypes in Canada were analyzed based on unweighted pair group method with arithmetic means clustering, using the complement of Dice coefficient calculated from 21 EST-derived simple sequence repeat markers. Six groups of *P. tritticina* isolates were defined based on their virulence to *Lr2a*, *Lr2c*, and *Lr17a* including group I (avirulent on *Lr2a*, *Lr2c*, and *Lr17a*), group II (avirulent on *Lr2a* and *Lr2c*, virulent on *Lr17a*), group III (avirulent on *Lr2a*, virulent on *Lr2c*, avirulent on *Lr17a*), group IV (avirulent on *Lr2a*, virulent on *Lr2c*, and virulent on *Lr17a*), group V (virulent on *Lr2a* and *Lr2c*, avirulent on *Lr17a*), and group VI (virulent on *Lr2a*, *Lr2c*, and *Lr17a*).



also analyzed with the MODECLUS procedure in SAS as described by Harder et al. (2001). The resulting plot of *K*-value versus the number of clusters showed two large regions of stability (K = 6–11 and K = 12–24) at two and three clusters, respectively (data not shown). Subsequently, clusters defined at the region of stability were analyzed in a CANDISC-CDA plot. The distribution of clusters of virulence phenotypes was similar to that obtained in the 2D-PCA plot (data not shown).

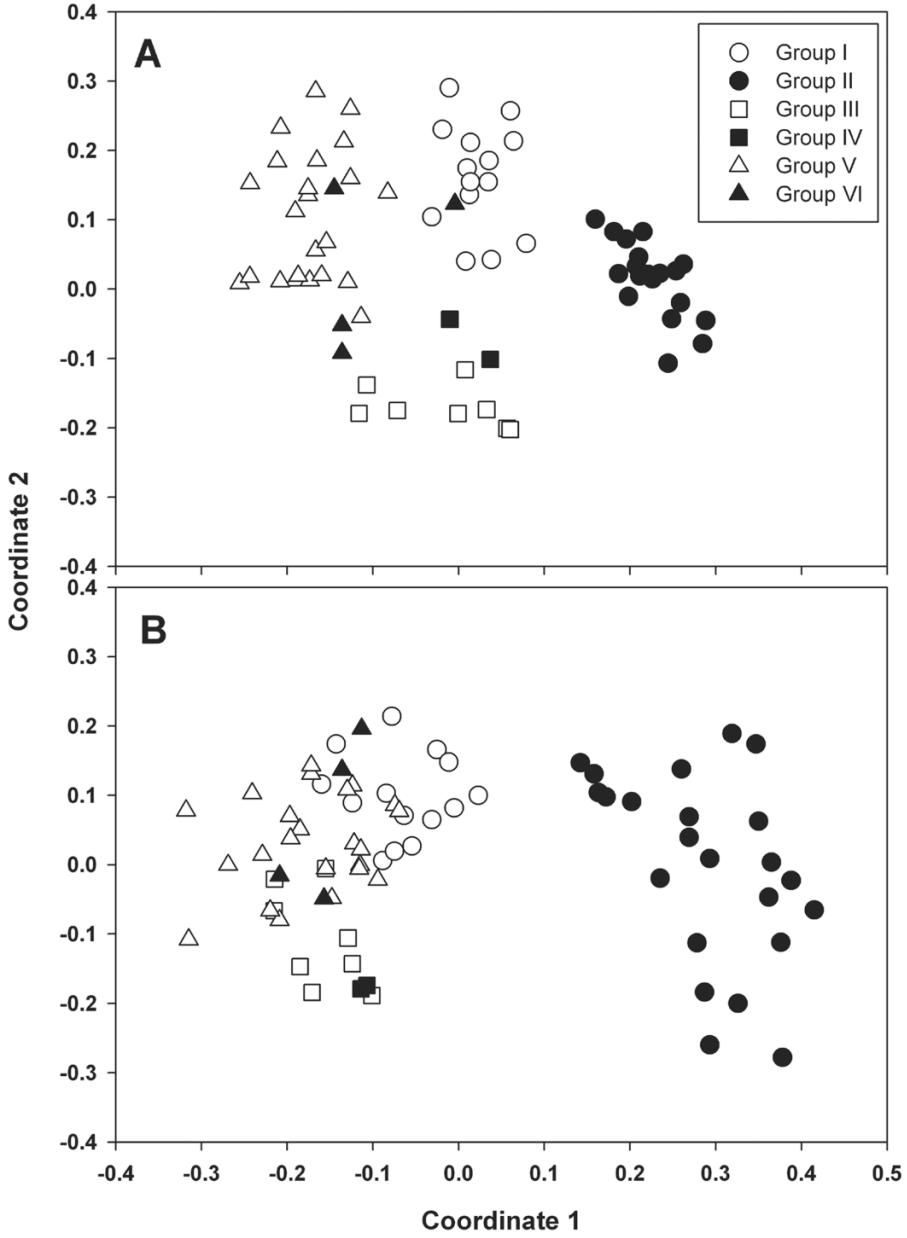
The representation of EST-SSR genotypes in a 2D-PCA plot was shown in Fig. 3B. The first two dimensions accounted for 76.7% of the variation. Virulence phenotypes in group II were separated from those in other groups in the plot. The clustering structure in the genetic dissimilarity matrix was also analyzed using MODECLUS. A large region of stability (K = 7–21) was found at two clusters with group II virulence phenotypes clearly separated from virulence phenotypes in group I, III, IV, V, and VI using the CANDISC-CDA analysis (data not shown).

The occurrence of *P. triticina* virulence phenotypes in Manitoba and Saskatchewan from 1997 to 2007 was analyzed. Virulence phenotypes in group II were most common in Manitoba and Saskatchewan from 1997 to 2001, accounting for 46%–61% of *P. triticina* isolates collected in the region (Fig. 4A) and MBDS was the predominant virulence phenotype (Table 3). Since 2001, virulence phenotypes in group V increased rapidly in frequency and constituted over 75% of the population in 2006 and 2007. Although TGBJ was predominant in 2001, it was replaced by TDBJ and TDBG from 2002 to 2007. TGBJ was virulent on *Lr16* but avirulent on *Lr24*, whereas TDBJ and TDBG were both avirulent on *Lr16* but virulent on *Lr24*.

Virulence phenotypes in Group III were the most common virulence phenotypes in Quebec and Ontario in 1997 and 1998 (Fig. 4B; Table 3). They were then replaced by virulence phenotypes in group II, which accounted for nearly 50% of *P. triticina* isolates collected in 2006 and 2007. Group III virulence phenotypes were also occasionally present in British Columbia and Alberta, but they were rare in Manitoba and Saskatchewan (Table 3).

Compared with the prairie and eastern regions of Canada, the size of *P. triticina* samples collected in British Columbia and Alberta was relatively small, which resulted in a higher sampling error and more erratic distribution of virulence

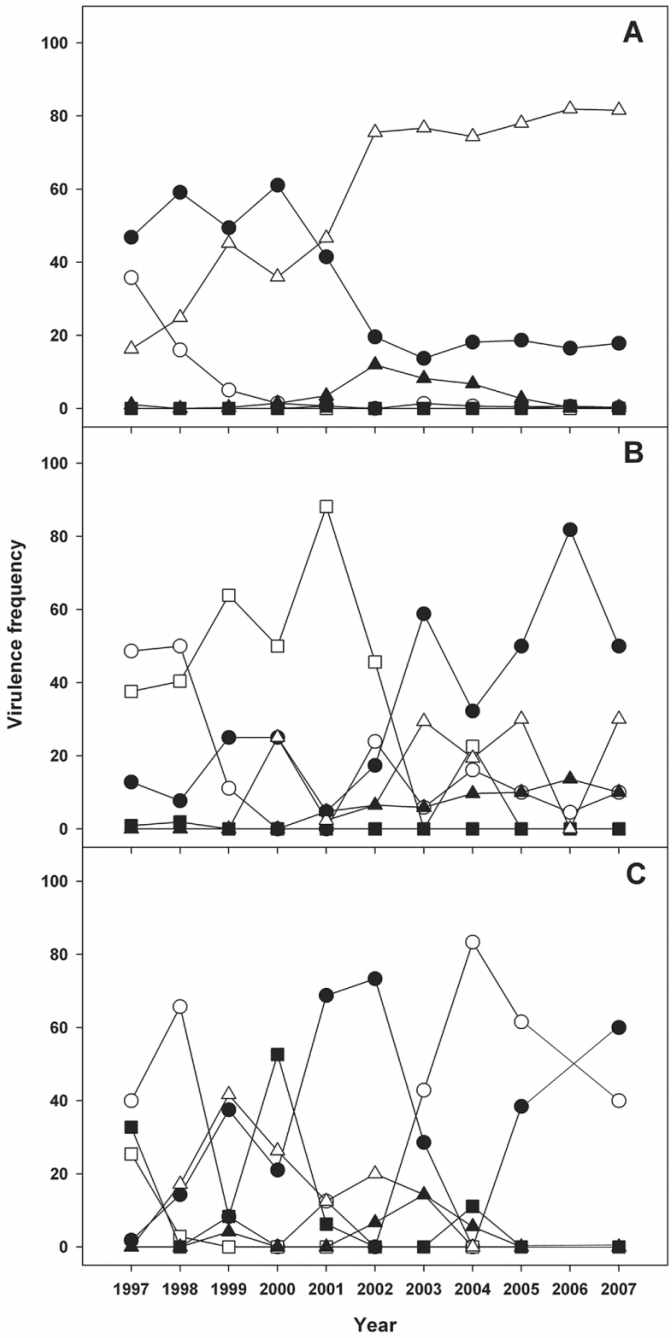
Fig. 3. Two-dimensional principal coordinate plot of *Puccinia triticina* isolates in Canada based on the simple dismatch coefficient derived from virulence (A) and the compliment of dice coefficient from 21 ESTs (B). Six groups were delimited by the following: open circles (○), group I (avirulent on *Lr2a*, *Lr2c*, and *Lr17a*); filled circles (●), group II (avirulent on *Lr2a* and *Lr2c*, virulent on *Lr17a*); open squares (□), group III (avirulent on *Lr2a*, virulent on *Lr2c*, and avirulent on *Lr17a*); filled squares (■), group IV (avirulent on *Lr2a*, virulent on *Lr2c* and *Lr17a*); open triangles (△), group V (virulent on *Lr2a* and *Lr2c*, avirulent on *Lr17a*); filled triangles (▲), group VI (virulent on *Lr2a*, *Lr2c*, and *Lr17a*).



phenotypes. In 1997, virulence phenotypes in group III and IV were common in this region, and the frequency of these virulence phenotypes decreased to less than 10% from 2000 to 2007 (Fig. 4C). Group I and group II virulence phenotypes were common in the region from 1997 to 2007, but the composition of virulence phenotypes changed annually (Table 3).

In Manitoba and Saskatchewan, virulence frequency to *Lr16* was at 16% in 1999 and increased to 74% in 2001, owing to the predominance of TGBJ (group V). It then decreased to less than 10% from 2004 to 2007 when TDBJ and TDBG, which were both avirulent on *Lr16*, became predominant (Fig. 5). From 1997 to 2004, virulence frequency to *Lr14a* and *Lr24* was relatively stable in the region (Fig. 5A). Since 2004, virulence frequency to *Lr14a* decreased to less than 40% with the increase of TDBG, which was avirulent on *Lr14a,* whereas virulence frequency to *Lr24* increased from 0% to over 70% when TDBG and TDBJ, both virulent on *Lr24*, became predominant. The positive non-random association between paired virulence to *Lr2a* and *Lr2c*, to *Lr3Ka* and *Lr30*, to *Lr17a* and *LrB*, and the negative non-random association between virulence to

Fig. 4. Frequency of *Puccinia tritticina* virulence phenotypes in Manitoba and Saskatchewan (A), Quebec and Ontario (B) and Alberta and British Columbia (C) from 1997 to 2007. Open circles (○), group I (avirulent on *Lr2a*, *Lr2c*, and *Lr17a*); filled circles (●), group II (avirulent on *Lr2a* and *Lr2c*, virulent on *Lr17a*); open squares (□), group III (avirulent on *Lr2a*, virulent on *Lr2c*, and avirulent on *Lr17a*); filled squares (■), group IV (avirulent on *Lr2a*, virulent on *Lr2c* and *Lr17a*); open triangles (△), group V (virulent on *Lr2a* and *Lr2c*, avirulent on *Lr17a*); filled triangles (▲), group VI (virulent on *Lr2a*, *Lr2c*, and *Lr17a*).



Lr2a and *Lr17a* were observed in Manitoba and Saskatchewan from 1997 to 2007 in the contingency table test of virulence (Table 4). The virulence diversity of the *P. triticina* population in Manitoba and Saskatchewan was relatively stable from 1997 to 2005. The Shannon and Simpson index varied from 1.5 to 2.1 and 0.6 to 0.8, respectively (Figs. 6A and 6B). Nevertheless, the Shannon and Simpson indexes were relatively low in 2006 and 2007 when TDBG and TDBJ became predominant and accounted for over 50% of the isolates collected.

In Quebec and Ontario, virulence frequency to *Lr16* was less than 16% from 1997 to 2007 (Fig. 5B). Virulence frequency to *Lr14a* fluctuated from 30% to 100% between 1997 and 2001 and stayed above 60% from 2002 to 2007. Virulence frequency to *Lr24* was less than 24% from 1997 to 2004 and increased to over 67% in 2006 and 2007. Although combined virulence to the gene pairs *Lr2a* and *Lr2c, Lr17a* and *LrB,* and *Lr30* and *Lr3ka,* were positively associated from 2003 to 2007 (Table 4), these associations of virulence are less accurate, owing to the relatively small size of samples collected in the region. The phenotypic diversity of *P. triticina* in Quebec and Ontario was relatively stable from 1997 to 2005, and the Shannon and Simpson indexes varied from 2.2 to 2.4 and from 0.8 to 0.9, respectively. In 2006 and 2007, the Shannon and Simpson indexes were significantly lower than in previous years when MFDS became predominant and accounted for nearly 75% of *P. triticina* isolates collected in the region (Figs. 6A and 6B).

In British Columbia and Alberta, virulence frequency to *Lr16* and *Lr24* was lower than 30% from 1997 to 2007 (Fig. 5C). Virulence frequency to *Lr14a* increased from 47% to nearly 100% between 1997 and 1999 and it was higher than 80% from 2000 to 2007. Although the positive non-random association between virulence to *Lr2a* and *Lr2c*, to *Lr17a* and *LrB,* and to *Lr30* and *Lr3Ka* were observed in 1999 and from 2001 to 2007 (Table 4), these associations may not be accurate, owing the small size of samples collected in the region. The diversity of virulence phenotypes in Alberta and British Columbia showed a decreasing trend from 1997 to 2002. The Shannon and Simpson indexes decreased from 2.1 to 0.9 and 0.8 to 0.5, respectively. In 2003, the diversity of virulence phenotypes increased to the level similar to that found in 1997 and then it declined from 2004 to 2007 (Figs. 6A and 6B).

To determine whether there were any significant correlations between virulence attributes and EST-SSR genotypes, dissimilarity matrices derived from virulence and EST-SSRs were compared for correlations using the Mantel coefficient in NTSYS 2.1. A significant correlation ($r = 0.46$, $p < 0.001$) was found between virulence to 16 'Thatcher' differential lines and EST-SSR genotypes. Dissimilarity matrices based on virulence to each of 16 wheat leaf rust differential lines, were also generated and compared for correlations with those generated from EST-SSRs. Correlations between EST-SSR genotypes and virulence to *Lr17a* ($r = 0.59$, $p < 0.001$), *Lr2a* ($r = 0.35$, $p < 0.001$), or *Lr2c* ($r = 0.45$, $p < 0.001$) were most significant. Fisher's exact test was also used to determine whether EST-SSRs were significantly associated with virulence to each of the 16 leaf rust resistance genes (Table 5). Seven out of 21 EST-SSRs showed significant association with virulence to *Lr2c* and *Lr17a* and six EST-SSR markers were associated with virulence to *Lr2a*. None of the EST-SSRs were associated with virulence to

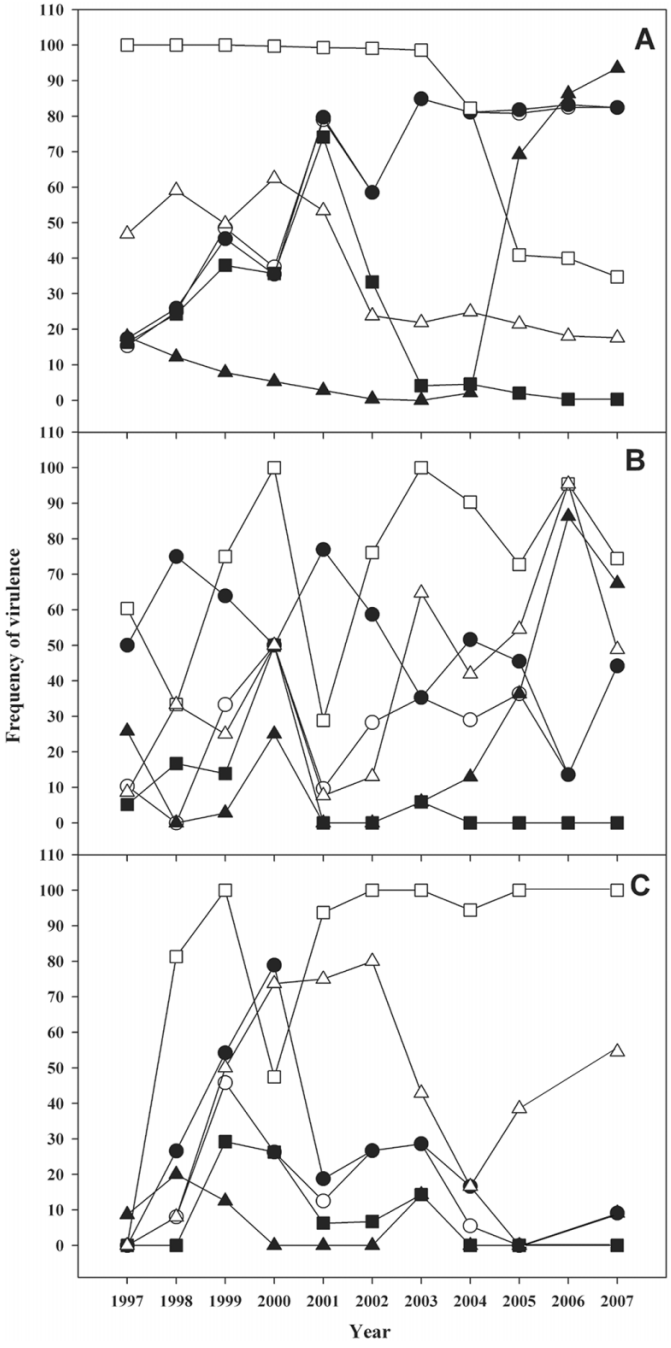
Table 3. Summary of predominant virulence phenotypes in Canada from 1997 to 2007 and groups based on virulence to *Lr2a*, *Lr2c*, and *Lr17a*.

Year	Most frequent virulence phenotypes	Group	No.[a]	2nd-most frequent virulence phenotypes	Group	No.	3rd-most frequent virulence phenotypes	Group	No.	Total number of isolates from survey
MB+SK[b]										
1997	MBDS	II	73	MBRJ	I	38	MDRJ	I	21	190
1998	MBDS	II	86	TGBJ	V	17	THBJ	V	15	181
1999	MBDS	II	148	TGBJ	V	55	THBJ	V	55	334
2000	MBDS	II	190	TGBJ	V	76	THBJ	V	19	339
2001	TGBJ	V	143	MBDS	II	55	THBJ	V	53	286
2002	MBDS	II	77	TGBJ	V	64	TBBJ	V	27	234
2003	TBBJ	V	45	MBDS	II	8	KBBJ	V	7	73
2004	TBBJ	V	135	TBBG	V	49	MBDS	II	28	281
2005	TDBG	V	185	TBBG	V	43	TDBJ	V	36	396
2006	TDBG	V	177	TDBJ	V	59	MFPS	II	17	315
2007	TDBG	V	197	TDBJ	V	54	MDPS	II	17	323
ON+QC										
1997	PBLQ	III	23	MDRJ	I	19	MBDS	II	9	115
1998	PBLQ	III	5	MGDS	II	2	MBDS	II	1	12
1999	MBDS	II	9	TBRJ	V	6	PCLR	III	4	36
2000	MBDS	II	4	TKBJ	V	2	THBJ	V	1	8
2001	PCLR	III	10	PBLR	III	8	PBLQ	III	5	53
2002	MBRK	I	7	PCLR	III	6	MBRJ	I	5	46
2003	MBDS	II	4	MBJS	II	3	MCJJ	II	2	17
2004	MBDS	II	5	MCJS	II	3	NBBT	III	3	31
2005	TDBG	V	2	MBBJ	I	1	MBJD	II	1	11
2006	MFDS	II	17	TBDS	VI	2	MBMR	I	1	22
2007	MLDS	II	13	TDBG	V	10	TDBJ	V	8	43
AB+BC										
1997	PBDB	IV	17	NBBR	III	13	MBGJ	I	8	57
1998	MBGJ	I	10	MCBJ	I	4	MBDS	II	3	35
1999	MBDS	II	9	TGBJ	V	5	PBDS	IV	2	24
2000	PBDG	IV	8	MBDS	II	4	TGBJ	V	3	19
2001	MBDS	II	11	THMJ	V	1	TBMJ	V	1	14
2002	MBDS	II	11	TBBJ	V	1	TBDS	VI	1	15
2003	MBBJ	I	2	MBDS	II	1	BBBN	I	1	7
2004	PBDG	IV	1	PBDK	IV	1	TBDJ	VI	1	3
2005	No samples			No samples			No samples			
2006	MBBJ	I	6	MBDS	II	3	MBGJ	I	2	13
2007	MBDS	II	4	MBGJ	I	2	MLDS	II	2	11

[a]No., number of isolates.

[b]MB+SK, Manitoba and Saskatchewan; ON+QC, Ontario and Quebec; AB+BC, Alberta and British Columbia.

Fig. 5. Frequency (%) of *Puccinia triticina* isolates collected in Manitoba and Saskatchewan (A), Quebec and Ontario (B), and Alberta and British Columbia (C) with virulence to 'Thatcher' wheat near-isogenic line for resistance gene *Lr2a* (open circles, ○), *Lr2c* (filled circles, ●), *Lr14a* (open squares, □), *Lr16* (filled squares, ■), *Lr17a* (open triangles, △), and *Lr24* (filled triangles, ▲) from 1997 to 2007.



leaf rust resistance genes *Lr3*, *Lr9*, *Lr16*, *Lr3Ka*, *Lr11*, *Lr30*, and *Lr10.*

Discussion

Kolmer (1999*b*) reported that the diversity of *P. triticina* virulence phenotypes in Manitoba and Saskatchewan was relatively stable from 1987 to 1997. The current study revealed that the virulence diversity fluctuated in this region from 1997 to 2007 (Fig. 6). The virulence diversity declined with the rapid increase of MBDS virulence phenotype in 2000 and 2001, when MBDS became the predominant virulence phenotype accounting for over 50% of the total population (Table 3). Similarly, a decrease in virulence diversity also occurred in 2006 and 2007 when TDBG and TDBJ became predominant and accounted for over 70% of the population. This decrease in the virulence diversity indicated that the directional selection of predominant virulence phenotypes had resulted in fewer virulence phenotypes and a less diverse population in Manitoba and Saskatchewan over time.

Virulence phenotype MBDS differed from TDBG and TDBJ in virulence on *Lr2a*, *Lr2c,* and *Lr24. Lr2a* is thought to be present in Canadian wheat cultivar 'Superb' (B.D. McCallum and P.L. Seto-Goh, unpublished data), which was first registered in 2000 and became relatively popular in 2006 and 2007 (McCallum and DePauw 2008). The replacement of MBDS by TDBG and TDBJ in Canada may have been partially driven by the selection on 'Superb'. On the other hand, TDBG and TDBJ are both virulent on *Lr24* but MBDS is not. Although *Lr24* is not thought to be present in most Canadian wheat cultivars (McCallum and DePauw 2008), it is commonly deployed in hard red spring wheats and breeding lines grown in the northern Great Plains of the United States (Kolmer 2005; Kolmer et al. 2007). It is possible that these virulence phenotypes were initially selected by virulence on *Lr24* and urediniospores of selected virulence phenotypes were then blown northward from the north-central United States into Manitoba and Saskatchewan.

Previous surveys in the United States and Canada showed that similar predominant virulence phenotypes were often selected by the host populations in the prairie region of Canada and the northern Great Plains region of the United States, regardless of the difference in leaf rust resistance genes deployed (Kolmer et al. 2005, 2007; McCallum and Seto-Goh 2002, 2005, 2008). Similarly, Kolmer (1993) found that the same predominant virulence phenotype was selected by wheat lines carrying different leaf rust resistance genes and susceptible wheat variety 'Thatcher' after 12 uredinial generations which suggested that the fitness difference between *P. triticina* isolates could be a major selective force. It is also possible that TDBG and TDBJ may have intrinsic fitness advantage over MBDS, which contributes to the predominance of these virulence phenotypes.

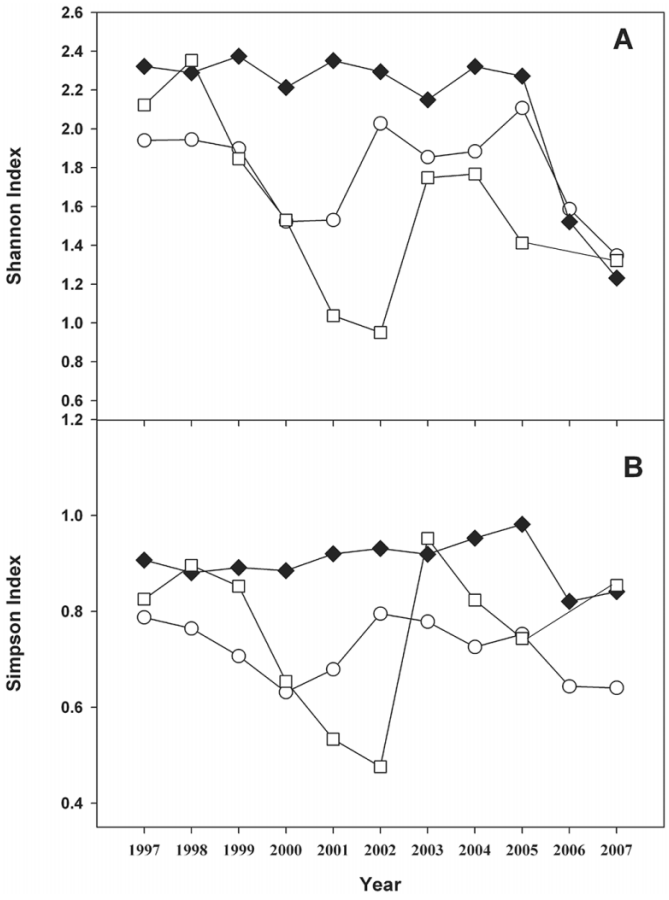
Some TDB_ virulence phenotypes, such as TDBG, had an unusual mesothetic reaction to *LrCen* and are also avirulent on *Lr14a* (Kolmer et al. 2006; McCallum and Seto-Goh 2008). Since virulence of *P. triticina* to *Lr14a* in North America has been fixed at nearly 100% with the cultivation of wheat cultivars with *Lr14a* (Kolmer 1991), Kolmer et al. (2007) suspected that these virulence phenotypes could be recently introduced, owing to their unique avirulence to *Lr14a.* In our study, two virulence phenotypes, TDBG and TDBJ, which differed in virulence on *Lr14a,* were placed in the same cluster based on either virulence or EST-SSRs. TDBG and TGBJ were closely related to virulence phenotypes in group V, which were virulent on *Lr2a* and *Lr2c* but avirulent on *Lr17a.* Similarly, Ordoñez and Kolmer

Table 4. Virulence associations to pairs of leaf rust resistance genes in *Puccinia triticina* population in Canada from 1997 to 2007 as measured by *G*-statistics.

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
AB+SK											
Lr2a, Lr2C	N.S.	N.S	+	N.S.	+	+	+	+	+		+
Lr3Ka, Lr30	N.S.	N.S.	+	N.S.	+	+	+	+	+		+
Lr17a, LrB	N.S.	N.S.	+	N.S.	+	+	+	+	+		+
Lr2a, Lr17a	N.S.	N.S.	–	–	N.S.	N.S.	–	N.S.	N.S.		N.S.
MB+BC											
Lr2a, Lr2C	+	+	+	+	+	+	+	+	+	+	+
Lr3Ka, Lr30	+	+	+	+	+	+	+	+	+	+	+
Lr17a, LrB	+	+	+	+	+	+	+	+	+	+	+
Lr2a, Lr17a	–	–	–	–	–	–	–	–	–	–	–
QC+ON											
Lr2a, Lr2c	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	+	N.S.	+	+	+
Lr3Ka, Lr30	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	+	+	+	+	+
Lr17a, LrB	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	+	N.S.	+	+	+
Lr2a, Lr17a	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Note: The *G* statistic is the contingency table test (Sokal and Rohlf 1981). –, significant negative association ($p < 0.001$); N.S., non-significant association ($p > 0.001$); +, positive association ($p > 0.001$). MB+SK, Manitoba and Saskatchewan; ON+QC, Ontario and Quebec; AB+BC, Alberta and British Columbia.

Fig. 6. Shannon (A) and Simpson indexes (B) of phenotype diversity of *Puccinia triticina* populations in Manitoba and Saskachewan (open circles, ○), Quebec and Ontario (filled squares, ■), and Alberta and British Columbia (open squares, □), from 1997 to 2007.



(2009) found that TDBG collected in the United States shared similar virulence and SSR genotypes with the group of *P. triticina* isolates that were virulent on *Lr2a* and *Lr2c* but avirulent on *Lr17a.* These results suggest that virulence phenotypes, which were avirulent on *Lr14a,* were most likely divergent from groups of virulence phenotypes pre-existing in the region (Kolmer et al. 2008). One possibility is that these virulence phenotypes were selected by their virulence on *Lr24*.

Virulence frequency to *Lr16* increased in 1999–2002 when TGBJ and THBJ, which were virulent on *Lr16*, became predominant. Virulence to *Lr16* also increased in the mid-1950s and early 1980s in North America with the introduction of wheat cultivars with *Lr16*, and subsequently declined to a very low level when winter wheat cultivars with *Lr16* were replaced (McCallum et al. 2007). This suggested that virulence to *Lr16* was under selection when this gene was deployed in the host population (Kolmer 1989, 1991). Interestingly, TGBJ and THBJ were replaced by TDBG and TDBJ, which only had an intermediate level of virulence on *Lr16*, in 2006 and 2007, despite the common usage of wheat cultivars with *Lr16* in North America. Currently, *Lr16* is present in several major Canadian wheat cultivars including 'AC Barrie', 'AC Domain', and 'AC Majestic' (McCallum et al. 2007). This recent change in virulence to *Lr16* indicates that virulence to *Lr16* alone does not confer enough selective advantage over other virulence phenotypes, and that other factors must also affect the selection of predominant virulence phenotypes.

Kolmer (1999*b*) reported that *P. triticina* isolates that were virulent on *Lr17a* had distinct virulence and genetic characteristics compared with isolates that were avirulent on *Lr17a*. It was hypothesized that this group of isolates was recently introduced in North America and selected by wheat cultivars in Kansas with *Lr17a*. In our study, virulence phenotypes in group II, which were avirulent on *Lr2a* and *Lr2c* but virulent on *Lr17a*, were placed in a distinct cluster in

Table 5. Association between simple sequence repeat markers and virulence of *Puccinia triticina* isolates from 1997–2007 differentiated based on one-sided Fisher's Exact Test ($p < 0.005$).

	Lr1	*Lr2a*	*Lr2c*	*Lr3*	*Lr9*	*Lr16*	*Lr24*	*Lr26*	*Lr3ka*	*Lr11*	*Lr17a*	*Lr30*	*LrB*	*Lr10*	*Lr14a*	*Lr18*
125	–	+	+	–	–	–	+	+	–	–	+	–	+	–	+	–
130	–	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–
182	+	+	+	–	–	–	–	–	–	–	+	–	+	–	+	–
3145	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	–
536	–	+	+	–	–	–	–	–	–	–	+	–	+	–	–	–
5594	–	+	+	–	–	–	–	–	+	–	+	–	+	–	+	+
639	–	+	+	–	–	–	–	+	–	–	+	–	+	–	+	–
5649	–	+	+	–	–	–	–	–	–	–	+	–	+	–	–	–

Note: –, non-significant association ($p > 0.05$); +, significant association ($p \leq 0.05$).

UPGMA analysis based on SSRs. Group II was also differentiated from the other groups in the principal coordinate analysis, based on either virulence or SSR markers. These results were in agreement with the previous study done by Kolmer (1999*b*), and suggested that group II virulence phenotypes could be recently introduced. Two virulence phenotypes from group IV (PBDK and PBDG) and four virulence phenotypes in group VI (TBDS, TBDJ, SBDJ, and SBDG) were also virulent on *Lr17a*. Nevertheless, PBDK and PBDG were clustered with virulence phenotypes in groups I and III in UPGMA and PCA analyses, whereas TBDS, TBDJ, SBDJ, and SBDG were closely related to those in group V, suggesting that these isolates were different from virulence phenotypes in group II despite their common virulence on *Lr17a*.

PBDK and PBDG were found in Quebec and Ontario, whereas TBDS, TBDJ, SBDJ, and SBDG were collected mainly in Manitoba and Saskatchewan. All of these virulence phenotypes only occurred at the low frequency. Kolmer (1992) reported that most Canadian *P. triticina* isolates collected in 1988 were heterozygous for virulence alleles to *Lr17a* based on their intermediate infection type. In this case, a single mutation was sufficient for avirulent *P. triticina* isolates to gain virulence against *Lr17a*. It is possible that these virulence phenotypes could emerge from pre-existing populations through mutation.

Park et al. (1999) attributed the origin of a new virulence phenotype in Australia to somatic recombination between two isolates in different groups of virulence phenotypes. We recently reported that nuclei from germ tubes of different *P. triticina* virulence phenotypes could be closely associated during the germ tube anastomosis when urediniospores from two virulence phenotypes were mixed and germinated (Wang and McCallum 2009). It is possible that some of the observed virulence phenotypes originated through the somatic hybridization between existing *P. triticina* virulence phenotypes. For example, two pre-existing virulence phenotypes that are both heterozygous for avirulence alleles can be associated and regrouped during the somatic hybridization of *P. triticina* leading to a homozygous virulent isolate. Somatic hybridization could also occur between virulence phenotypes from different groups e.g., between group II and group I, III, or V.

Since 2005, the frequency of virulence phenotypes in groups III and IV decreased rapidly in Ontario and Quebec. Only a single isolate within the group III virulence phenotype was found in the region in 2008 (B.D. McCallum and P.L. Seto-Goh, unpublished data). This decline in the frequency of group III and IV virulence phenotypes in Ontario and Quebec was unusual, since this group of virulence phenotypes had been common in this region since the mid-1950s. Although the absence of groups III and IV virulence phenotypes in recent years could be merely reflective of the small number of samples collected in the region (McCallum and Seto-Goh 2005, 2006, 2008), we cannot rule out the possibility that they are being replaced by other groups. In 2006 and 2007, most *P. triticina* isolates collected in Quebec and Ontario were virulent on *Lr24*. *Lr24* was likely present in one popular winter wheat cultivar in Ontario, 'Vienna' (B.D. McCallum and P.L. Seto-Goh, unpublished data), and possibly the other cultivars. The *P. triticina* pop-

ulation in this region could be under selection for virulence to *Lr24*. In addition, virulence phenotype MFDS was common in both Canada and the northern states of the United States in 2005 and 2006 (Kolmer et al. 2008; Kolmer et al. 2007), which suggested that this region received inoculum from nearby areas.

The number of *P. triticina* samples collected in Alberta and British Columbia was smaller compared with those collected in the prairie and eastern regions of Canada, which made it difficult to determine whether there was any significant shift in virulence in this region. Although most virulence phenotypes found in Alberta and British Columbia were also present in Manitoba and Saskatchewan suggesting a common source of inoculum between these two regions, several phenotypes collected in Alberta and British Columbia were not found in the much larger sample collections from Manitoba and Saskatchewan. Additionally, the rapid increase in virulence to *Lr2a*, *Lr2c*, and *Lr24*, which occurred in Manitoba and Saskatchewan since 2001, was not observed in Alberta and British Columbia. These differences indicate that the populations in Alberta and British Columbia are somewhat different from that in Manitoba and Saskatchewan. One explanation for this is that *P. triticina* inoculum from the northwestern United States spreads northwards into Alberta and British Columbia, in addition to inoculum from the Great Plains.

In summary, the clustering of *P. triticina* virulence phenotypes in Canada showed a strong correlation with virulence to *Lr2a*, *Lr2c*, and *Lr17a* despite the significant shift in virulence of *P. triticina* across Canada. Groups of *P. triticina* virulence phenotypes differentiated based on virulence to *Lr2a*, *Lr2c*, and *Lr17a*, have distinct genetic backgrounds as determined by SSR markers (Fig. 2). Assuming asexual reproduction in the North-American and Canadian *P. triticina* populations, this indicates that *P. triticina* is capable of maintaining distinctive molecular and virulence characteristics in each group. These results agree with those from other plant pathogens that have specialized physiological forms and reproduce asexually (Zeigler et al. 1995). Thirteen out of 21 EST-SSRs showed no association with virulence but 6 EST-SSRs were associated with virulence to *Lr2a*, *Lr2c*, and *Lr17a*. The differentiation of *P. triticina* virulence phenotypes in Canada was affected by both the introduction of foreign isolates with different molecular background and selective effects from the host population.

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