Population structure of *Puccinia striiformis* f. sp. *tritici*, the cause of wheat stripe rust, in western Canada

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By

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ABSTRACT

Stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most devastating diseases of wheat worldwide. Selection pressure on the pathogen population may result in a rapid shift to races virulent on wheat genotypes with specific resistance genes. For successful stripe rust management, it is important to monitor the virulence spectrum of the pathogen to detect new races. The purpose of this research was to survey Saskatchewan fields to determine the prevalence of stripe rust, characterize the race structure of *Pst* in western Canada and to determine the genotypic diversity of the pathogen population. Race characterization was performed by inoculating 27 near-isogenic wheat lines carrying 28 known resistance genes, four supplemental cultivars and one triticale cultivar with 61 genetically uniform *Pst* isolates from western Canada. Whole genome sequencing of pathogen isolates was conducted, using the Illumina HiSeq2500 platform and polymorphisms were assessed by single nucleotide polymorphism (SNP) variants. Characterization of *Pst* isolates identified 33 races of the pathogen. Genes *Yr5, Yr15* and *YrSP* conditioned resistance against all isolates tested and all isolates were virulent on *Yr6*, *Yr7*, *Yr9*, *Yr18, Yr28*, *Yr29* and *Yr31*. Variation for virulence was observed among isolates on *Yr10*, *Yr24*, *YrTye*, *YrSu*, *Yr3* and *Yr4*. The analyses of virulence profiles divided the 61 isolates into four sub-populations or groups. These four sub-populations were distinct from each other in terms of virulence spectrum and year of collection. The *Pst* population in Alberta had greater diversity in terms of virulence compared with the Saskatchewan population. Diversity at the genome level was not observed to be related to geographic location or virulence phenotypes of the isolates. The SNP data revealed four sub-populations in the western Canadian *Pst* population. Genomic analyses of 48 *Pst*

isolates did not reveal any relationship of the four sub-populations with their origin or year of collection. Signs of recombination were detected in the *Pst* population in western Canada. Genomic analyses differentiated isolates showing signs of recombination from those that did not.

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DEDICATION

I dedicate this thesis to all Punjab farmers, including my loving father

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LIST OF ABBREVIATIONS

AB	Alberta
AFLP	Amplified Fragment Length Polymorphism
APR	Adult Plant Resistance
BC	British Columbia
BIC	Bayesian Information Criterion
CYR	Chinese Yellow Rust
C-PST	Canada-*Puccinia striiformis* f. sp. *tritici*
DAPC	Discriminant Analysis of Principal Components
DNA	Deoxyribo Nucleic Acid
dpi	Days post inoculation
E	Evenness index
G	Gleason richness index
INDEL/indel/InDel	Insertion-deletion
KB	Kosman distance between regions
KW_m	Kosman diversity within population
MB	Manitoba
NGS	Next Generation Sequencing
NTSYS	Numerical taxonomy and multivariate analysis system
PNW	Pacific North-West
PHI	Pair-wise Homoplasy Index
Pst	*Puccinia striiformis* f. sp. *tritici*
RAPD	Random Amplified Polymorphic DNA
SAHN	Sequential, Agglomerative, Hierarchical, and Nested
SH	Shannon index
Sh	normalized Shannon index
Si	Simpson index
SK	Saskatchewan
St	Stoddart index

SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
STR	Short Tandem Repeat
UV	Ultraviolet
VAT	Virulence Analysis Tool

CHAPTER 1. INTRODUCTION

The world's population is expected to reach 7.9 billion by the year 2025, which will require production of approximately 786 M tonnes to feed this huge population (Curtis, 1982). Wheat production can be increased either by increasing the area in production or increasing the yield per unit area. There is limited scope for increase in the production area under wheat. Therefore, to increase yield per unit area there is a need to manage diseases and insect-pests of wheat that cause yield and quality losses worldwide.

Wheat is one of the most common crops grown in Canada. The wheat growers on the Canadian Prairies produce about 90% of the wheat in Canada (Fig. 1.1) (Anonymous, 2010b). Canadian wheat is in great demand in other countries due to its desirable milling and baking qualities (Curtis, 1982).

Figure 1.1. Wheat producing areas in western Canada (Anonymous, 2010c, Canadian Grains Commission).

Stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici (Pst)*, is one of the most important diseases of wheat and occurs everywhere the crop is grown (Roelfs *et al*., 1992). The geographical distribution of stripe rust is showed in the Fig. 1.2 (Stubbs, 1985; Chen, 2005). The disease causes reduced yield, quality and vigor of the produce (Chen, 2005). On susceptible varieties, disease development at the seedling stage can cause 100% yield loss (Chen, 2005). Mutation, selection pressure on mutants, somatic recombination and sexual recombination give rise to new pathotypes or races of the pathogen in a matter of years. Mutation and selection pressure are the most common cause of variation in *Pst* populations.

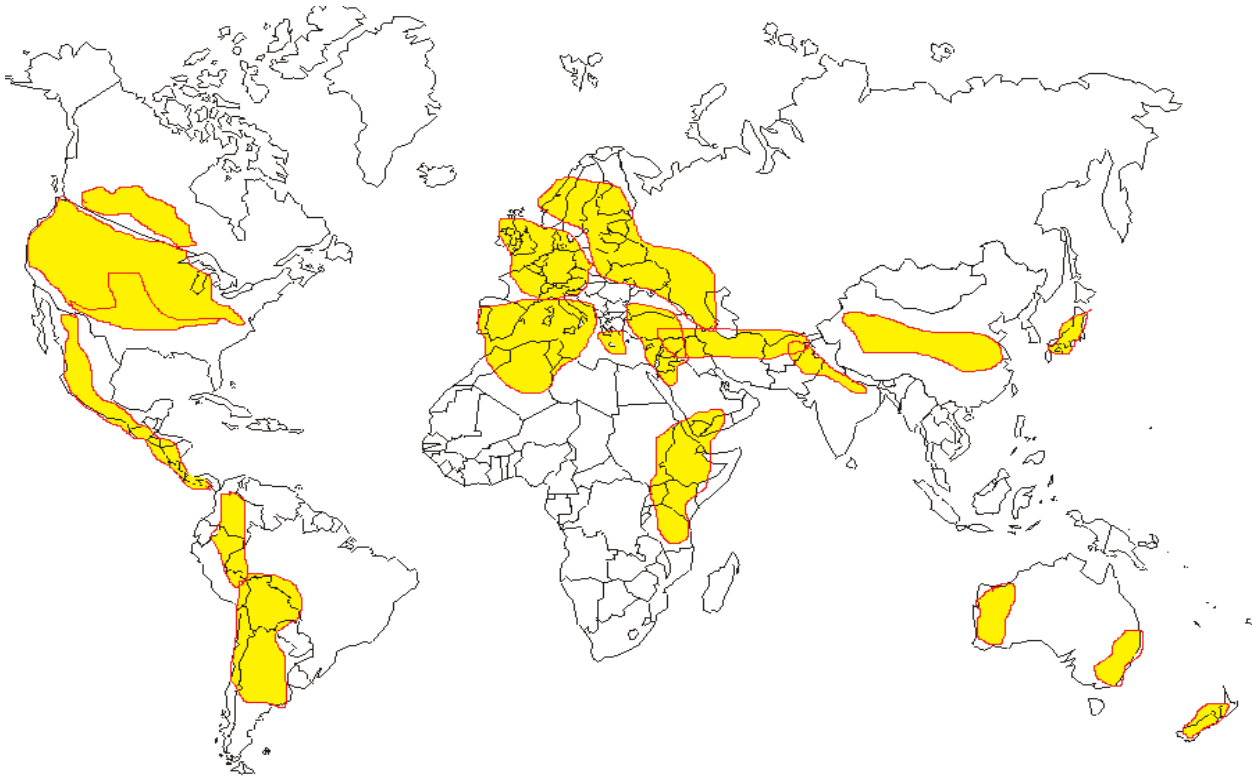


Figure 1.2. Worldwide geographical distribution of stripe rust.

There are two main practices that can be employed to manage stripe rust of wheat: the use of fungicides and genetic control through host resistance. Fungicides are effective, but expensive and environmentally unfriendly. The management of the disease with host resistance, i.e. resistant varieties, is effective and environment friendly (Chen, 2005). Different types of resistance such as seedling or all-stage resistance, adult plant resistance (APR) and high temperature adult plant resistance (HTAP) are common in wheat germplasm. However, varieties with a specific resistance gene usually remain effective only for a few years because the extreme selection pressure on the pathogen population with mutants results in a gain in virulence in the pathogen population for that particular gene. This is known as the 'boom and bust' cycle (Fig. 1.3). Quantitative/polygenic resistance is believed to be difficult to breakdown by the pathogen (Chen, 2013; Knott, 1989).

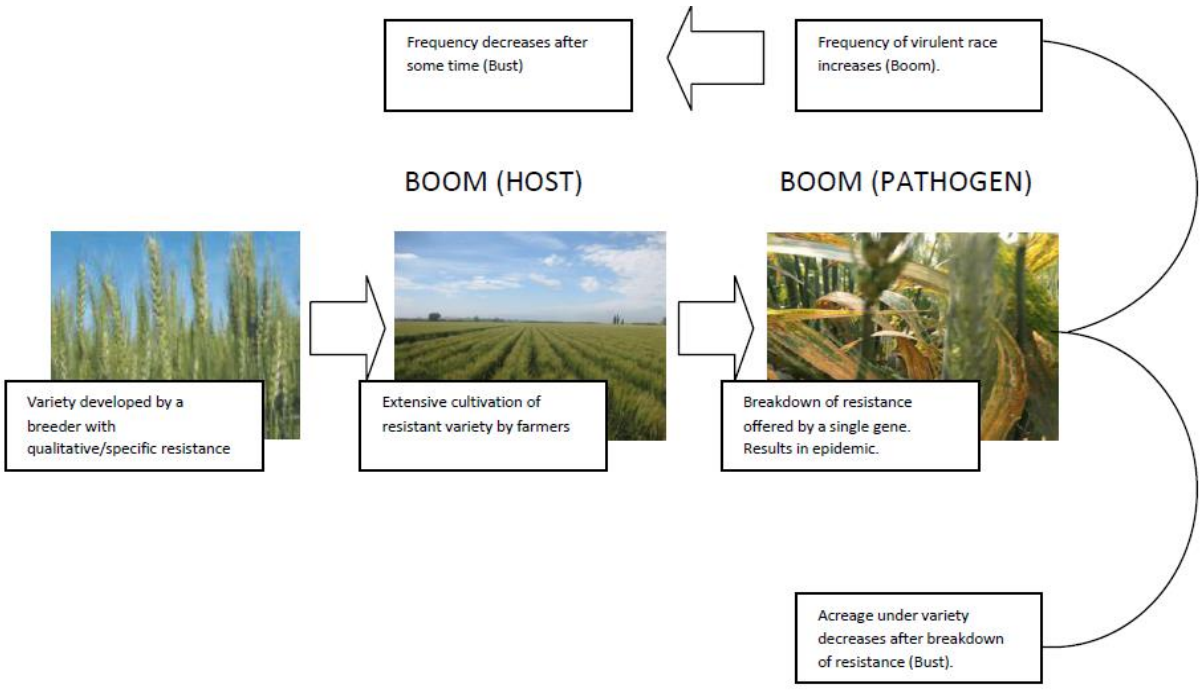


Figure 1.3. The 'Boom and Bust' cycle (Priestley, 1978).

The *Pst* pathogen is adapted to cool climatic conditions but a few studies in the recent past by Milus *et al*. (2006, 2009) suggest a change in the pathogen and the emergence of new races after the year 2000 that are adapted to warmer climatic conditions. Also the geographic range of the pathogen has expanded in the US when a new invasive population of the pathogen entered the USA around the year 2000 (Milus *et al*., 2009).

Effective use of resistance genes and sources of resistance, by plant breeders, requires knowledge of the virulence spectrum of this pathogen. The present study aims to understand virulence and genotypic variation in the *Pst* population prevalent in western Canada, and Saskatchewan in particular. The results from this study will assist breeders in making decisions on the selection of resistance sources and the development of resistant varieties. Knowledge of genotypic variation will facilitate an understanding of the epidemiology and population structure.

1.1. Project hypotheses

1. Variation for virulence exists in the *Pst* population on the Canadian prairies. Some virulence groups may occur more frequently than others during the cropping season. Also it is hypothesized that the population structure in Saskatchewan is different from that in Alberta and British Columbia as races here could be a mixture of inoculum from the Pacific North West (PNW) of the USA and from the south central USA arriving by way of the 'Puccinia Pathway'.

2. Changes in the genetic make-up of the population leads to changes in virulence and adaptation. Determining the genetic make-up of the population can facilitate an understanding of the pathogen.

1.2. Project objectives

The objectives of this project were to examine virulence and genetic variation in the *Pst* population of western Canada, particularly Saskatchewan. Corresponding to the above mentioned hypotheses, the following were the objectives of this thesis:

1. to survey wheat crops in the Saskatchewan to determine disease distribution in Saskatchewan and collect *Pst* isolates,

2. to characterize the race structure of the Saskatchewan population of *Pst* collected in 2011-2013, and

3. to determine the genetic diversity of *Pst* in Saskatchewan and compare with the population in neighboring provinces using next-generation sequencing (NGS) technology and SNP variants to relate molecular genotypes with virulence phenotypes, geographical location of different races and year of collection.

CHAPTER 2. REVIEW OF LITERATURE

2.1. Wheat (*Triticum* spp.): The King of Cereals

2.1.1. Production in Canada and World

Canada is among the top exporters of wheat with production of approximately 30.6 M tonnes in 2013 (Anonymous, 2014a). In 2013, Saskatchewan produced approximately 14.5 M tonnes of wheat, Alberta 9.4 and Manitoba, 4.1. Wheat has been an important Canadian crop since its introduction by the first European settlers. It was cultivated on approximately 24 million acres in Canada in 2014. Every year, Canada exports approximately 20 M tonnes of wheat and flour, thus making it the second largest export country in the world (Menzies and Gilbert, 2003).

2.1.2. Economic importance

Wheat is one of the most important cereal crops in the world and serves as a staple food for millions of people worldwide (Curtis, 2002). Wheat in Canada is used for making noodles, pasta, and many kinds of bread, as well as feed for livestock (Menzies and Gilbert, 2003). Canadian wheat is high in protein and has excellent baking quality. For these reasons, there is great demand for wheat in other countries (Curtis, 1982). Wheat is also used to make beer and feed livestock (Anonymous, 2010c, Canadian Grains Commission). It has the potential to be used as a bio-fuel crop as well.

2.1.3. Taxonomy & biology

Wheat is a monocot in the family Poaceae, whose center of origin is the Fertile Crescent region between the Tigris and Euphrates rivers (Sharma, 2012). Wheat (*Triticum* spp.)

cultivation is as old as the history of crop domestication, approximately 8000 years (www.fao.org). The majority of wheat grown in the world and Canada is bread wheat (*Triticum aestivum* L. em Thell.). Another wheat species commonly cultivated in Canada is *Triticum turgidum* spp. *durum* (durum or pasta wheat). Wheat has three ploidy levels: diploid (einkorn wheat), tetraploid (durum, emmer, rivet, Polish and Persian wheats), and hexaploid (bread or common, club, spelt and shot wheats). Durum (*T. turgidum* spp. *durum*) and bread wheat (*T. aestivum*) are the most common (Knott, 1989). Bread wheat is an annual grass and the varieties grown in Canada are mainly spring type, although a small percentage (~5%) is winter wheat (Anonymous, 2014b).

2.2. Stripe rust of wheat: the causal pathogen, history and importance of the disease

Stripe rust is caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., (abbreviated as *Pst*) and is prevalent throughout the world, wherever wheat is cultivated (Markell and Milus, 2008; Line, 2002; Chen, 2005; Wellings, 2011), in temperate-cool and wet environments (Yahyaoui *et al*., 2002; Rapilly, 1979). Stripe rust is not new and likely occurred even before the domestication of wheat (Curtis, 1982). Gadd described stripe rust for the first time in 1777 (Chen, 2005). *Puccinia striiformis* was considered the causal agent for an epidemic in rye crops in Sweden in 1794 (Stubbs, 1988). The cereal rust fungus *Puccinia striiformis* in the family *Pucciniaceae* belongs to the order Pucciniales, formerly Uredinales, of the Phylum Basidiomycota and class Basidiomycetes (Hibbett *et al*., 2007). This fungus causes stripe rust of various cereal crops and grasses. It was given various names such as *Uredo glumarum*, *Puccinia striaeformis*, *Puccinia stramini*, and *Puccinia glumarum* (Stubbs, 1985) until its current name *Puccinia striiformis* was suggested in 1953 (Hylander *et al*., 1953

cited in Line, 2002; Stubbs, 1985). The current scientific name *Puccinia striiformis* was assigned by Hylander and co-workers in 1953 and later reviewed by Cummins & Stevenson (1956). Humphrey *et al*., (1924) called the disease 'stripe rust' and the name 'yellow rust' was suggested by Eriksson and Henning in 1894 (Stubbs, 1985). Eriksson and Henning (1896 cited in Line, 2002) provided a detailed account of stripe rust nomenclature and history in a monograph on cereal rusts. Cummins and Stevenson called the pathogen *P. striiformis* West. (*P. striiformis* Westend.) in 1956 (Manners, 1960).

Stripe rust has been reported from about 60 countries and on all continents except Antarctica (Chen, 2005). Stripe rust of wheat is the most important rust pathogen of wheat worldwide and causes huge losses (up to 70%) every year. The areas in the world prone to serious damage by this pathogen are the USA (Pacific Northwest in particular), East Asia (China North-West and South-West), South Asia (India, Pakistan and Nepal), Australia, New Zealand, East Africa (Ethiopia, Kenya), the Arabian Peninsula (Yemen) and Western Europe (East England) (Wellings, 2011). The disease is considered to be endemic in central and west Asia (Yahyaoui *et al*., 2002).

The genus *Puccinia* is the most economically important of the *Pucciniaceae* family and consists of more than 3,000 species. Different *formae speciales* of *Puccinia striiformis* Westend. cause stripe rust of cereals and grasses (Chen, 2005). All species of *Puccinia* differ in terms of host preference and number of spore stages in their life cycle (Liu and Hambleton, 2010; van der Merwe *et al*., 2007). Nomenclature of the stripe rust fungus was recently reviewed by Liu and Hambleton (2010) based on DNA sequence polymorphism. They reported four different lineages (*P. striiformis, P. striiformoides*, *P. pseudostriiformis, P.*

gansensis) of *Puccinia striiformis* called '*Puccinia* series *striiformis'* and all these are considered different species as they have different morphological features such as size of urediniospores, surface echinulation, number of germ pores, hilum width of teliospores, and other morphological characteristics (Liu and Hambleton, 2010).

Urediniospores and teliospores of the fungus are dikaryotic, and teliospores produce haploid basidiospores (Chen, 2005). Pycnial and aecial spore stages of the fungus were confirmed recently (Jin *et al*., 2010). There are six chromosomes in haploid nuclei of *Puccinia striiformis* (Goddard, 1976). Chromosome size in metaphase nuclei, 0.7-1.1 µm, is similar to *P. recondita* and *P. graminis* (Goddard, 1976).

Extensive studies on specialization and changes in virulence of *Pst* have been conducted in Europe (Zadoks, 1961), the UK (Manners, 1950), Canada (Newton and Johnson, 1936) and the western USA (Purdy and Allan, 1963). Specialization between host genera and within a single host at the genotype level exists in *Pst*. Specialization on different host genera means the pathogen varies in infective and reproductive capacity. On this basis, Erikkson in 1894, recognized five special forms (*formae speciales*) of the pathogen. These were: *P. striiformis* f. sp. *tritici* on wheat, *P. striiformis* f. sp. *hordei* on barley, *P. striiformis* f. sp. *secalis* on rye, *P. striiformis* f. sp. *elymi* on *Elymus* spp. and *P. striiformis* f. sp. *agropyron* on *Agropyron* spp. (Hovmøller *et al*., 2011). One more special form of *Puccinia striiformis*, which attacks wild barley (*Hordeum* spp.) is designated as *Puccinia striiformis* f. sp. *pseudo-hordei* (Wellings, 2000).

Ravn and his associates first identified stripe rust on various wheat cultivars in North America in 1915 (Carleton, 1915; Humphrey *et al*., 1924; Humphrey *et al*., 1916; Line,

2002). Stripe rust was not regarded as an important disease in the US until the 1960s, when severe epidemics occurred in California and the Pacific Northwest (Shaner *et al*., 1973; Tollenaar *et al*., 1967). In Canada, stripe rust was first observed on Vancouver island in British Columbia in 1916 on grasses (Line, 2002) and in Alberta in 1918 on *H. jubatum* (Fraser and Conners, 1925; Line, 2002) and again in Alberta in 1926 (Johnson and Newton, 1928). The area of most concern was southern Alberta, as this was presumed to be the area of survival of *Pst* and stripe rust was as a major wheat production problem in southern Alberta (Sanford and Broadfoot, 1932; Conner *et al*., 1988). In Saskatchewan, stripe rust was first observed in 1928 (Line, 2002), and in later years was reported in Alberta, British Columbia, southern Alberta and western Saskatchewan, but not in Manitoba or eastern Saskatchewan (Newton and Johnson, 1936). The disease at that time was most common on native grass species in southwestern Saskatchewan, Alberta and British Columbia (Stubbs, 1985) and stripe rust from *H. jubatum* and *Agropyron* species attacked wheat (Line, 2002).

Until 2000, workers in Canada did not consider stripe rust an economically important disease of wheat (Line, 2002). Although it was a major concern to wheat under irrigation in southern Alberta, it was absent in eastern Saskatchewan and Manitoba (McCallum *et al*., 2003, 2004). Stripe rust appeared in 2000 in the eastern parts of the Canadian prairies (McCallum and Fetch, 2001) and the virulence spectrum of the pathogen was wider than it was before 2000 (Su *et al*., 2001). In western Canada, it was Sanford (1932) and Broadfoot (1933) who first studied the epidemiology and distribution of stripe rust (Su *et al*., 2003). Since 2000, the disease has become a regular pest of wheat in Saskatchewan, as well as the eastern provinces of Canada (McCallum *et al*., 2003, 2004).

2.2.1. Losses and epidemics

Rust diseases of field crops (wheat in particular) cause large crop losses every year (Fetch *et al*., 2011). The importance of rust diseases vary in cultivated wheat depending on environmental conditions, inoculum levels and susceptible host varieties (Wellings, 2011). Stripe rust affects quality and yield of the produce. Seed obtained from stripe rust infected field crops has reduced vigor and poor germination. Even total yield loss is not uncommon in areas where the disease starts early in the season and continues to develop for several months. Stripe rust can cause up to 70% yield loss, although loss varies depending on host resistance, time of initial infection, rate of disease development and disease duration. Losses of up to 20% (Doling and Doodson, 1968) and 75% (Roelfs, 1978) in wheat were reported in the USA. In 1937, Bever reported 65% yield loss in an experiment where a susceptible cultivar in the greenhouse was inoculated with stripe rust (Wellings, 2011). Yield losses of 20% were reported by Purdy and Allan (1963) when the spikes of a cultivar with resistance were infected. Pandemics of wheat stripe rust in the 1970s in North Africa, the Indian subcontinent, the Middle East, the East African Highlands and China (Saari and Prescott, 1985) occurred because the *Yr2* gene, which was present in most of the cultivars at that time, was defeated (McIntosh, 2009). Susceptible cultivars and favorable weather resulted in the first epidemic in South Africa in 1996 (Pretorius, 1996). Epidemics were again observed in South Africa in 1997 and 1998. Losses of nearly US$ 2.25 million were calculated in the 1998 epidemic that occurred in the eastern Free State of South Africa (Pretorius, 2004). In 2002, an epidemic in China on 66 million ha of wheat resulted in a yield loss of 13 M tonnes (Wan *et al*., 2004). Stripe rust caused approximately 20-40% losses in 1999 and 2000 in central Asia (Morgounov *et al*., 2004). In Australia, AU$ 40

million was spent on crop protection to control the 2003 epidemic of stripe rust (Wellings and Kandel, 2004). The most damaging stripe rust epidemic in the USA was in 2000, when the disease appeared in at least 20 states of the USA (Markell and Milus, 2008). This epidemic of stripe rust was a record resulting in the loss of more than 9 M bushels of wheat from eight states of the USA (Chen *et al*., 2010).

Stripe rust was reported to cause losses in wheat yield and a reduction in grain quality in soft white spring wheat in Alberta during the 1980s (Conner *et al*., 1988). Stripe rust epidemics in the past few years in Canada indicated that the disease can cause significant losses in the Canadian prairies (Kumar *et al*., 2012). Susceptible cultivars and a favorable environment are required for stripe rust development. Epidemics of wheat stripe rust were reported in the 1990s in central Alberta. An epidemic in a wheat nursery in 2005 caused 100% loss (McCallum *et al*., 2006) and another was reported in central Saskatchewan in 2006 (McCallum *et al*., 2007c). Stripe rust was common, but at low levels in Saskatchewan in 2013, although a number of severely infected fields were detected (Brar *et al*., 2014). Yield losses up to 35% were recorded on susceptible varieties in the 2011 stripe rust epidemic (Kutcher *et al*., 2012). The stripe rust pathogen has the potential to significantly reduce revenue to producers, even at a low percentage yield loss per acre. For example, in 2010 wheat production was estimated to be over 23 M tonnes. Even a 5% reduction in yield across Canada due to stripe rust would mean a reduction of over 1 M tonnes.

2.2.2. Host range

Puccinia striiformis f. sp. *tritici* is an obligate parasite of plants belonging to the Gramineae (Poaceae) family. Wheat and barley are the main hosts of *P. striiformis* (Hassebrauk, 1965).

Stripe rust infects about 320 grass species (artificially or naturally inoculated) belonging to 50 genera (Hassebrauk, 1965) in the subfamilies *Festucoideae* and *Eragosteae* (Gould, 1968). *Aegilops*, *Agropyron*, *Bromus, Elymus*, *Hordeum* and *Triticum* are major genera affected by stripe rust (Hassebrauk, 1965). There are several wild grass species that act as collateral hosts of stripe rust (Table 2.1), but their role in the epidemiology of the disease differs in different wheat growing areas of the world (Stubbs, 1985). In California and the Pacific Northwest (PNW) of the USA, wild grasses in the mountains serve as an oversummering source for *Pst* and this initiate infection on wheat plants at lower elevations (Hendrix *et al*., 1965; Tollenaar and Houston, 1967). However, wild grasses are of little importance in the epidemiology of the disease in Montana (Sharp and Hehn, 1963) and in northern China (Stubbs, 1985).

Table 2.1. Host range of *Puccinia striiformis* f. sp. *tritici* (*Pst*).

Primary hosts	Pycnial/aecial (alternate) hosts	Accessory hosts
Triticum[4] spp. i.e. cultivated wheat crops (*T. aestivum* L., *T. turgidum* var. *durum* L., *T. dicoccum* Schrank, *T. dicoccoides* Korn)	*Berberis* spp. (*B. atrocarpa*[1], *B. stenostachya*[1], *B. soulieana*[1], *B. shensiana*[1], *B. wangii*[1], *B. phanera*[1], *B. davidii*[1], *B. poiretii*[1], *B. aggregata* var. *integrifolia*[1], *B. potaninii*[1], *B. jamesiana*[1], *B. ferdinandi-coburgii*[1], *B. brachypoda*[1], *B. circumserrata*[1], B. *platyphylla*[1], *B. dasystachya*[1], *B. aggregata*[1], *B. chinensis*[2], *B. holstii*[2], *B. koreana*[2], *B. vulgaris*[2] and *B. guizhouensis*[1])	*Elymus canadensis*[4] L. *Leymus secalinus*[4] Hochst, *Agropyron*[4] spp. Garetn, *Hordeum*[4] spp. L. *Phalaris*[4] spp. L. and *Bromus unioloides*[4] Kunth (Pasture grasses)
Hordeum vulgare[4] L. (cultivated barley)	*Mahonia aquifolium*[3] (Oregon grape) under artificial inoculation	
Triticosecale[4] (Triticale)		
Secale cereale[4] L. (cultivated rye)		

[1]Zhao *et al*., 2013, [2]Jin *et al*., 2010, [3]Wang and Chen, 2013, [4]Chen *et al*., 2014

2.2.3. Disease symptoms and signs on the host plants

2.2.3.1. Disease symptoms and signs on wheat and grasses

Puccinia striiformis f. sp. *tritici* attacks green portions of cereals (wheat, barley, triticale and rye) and grass plants. Plants may become infected at any growth stage, from crop emergence to maturity. Under favorable environmental conditions, the pathogen takes one week to cause visible symptoms and about two weeks for sporulation after pathogen penetration of the host. On adult plants the fungus produces stripes of pustules consisting

of golden yellow to orange colored uredinia (Fig. 2.1a-c) on leaves along veins (Fig. 2.1d) (Chen, 2005; Line 2002; Knott, 1989). On seedlings, the stripes are not restricted by leaf veins and uredinia can cover the whole leaf area (Fig. 2.1b) (Line, 2002; Knott, 1989). The symptoms appear as very small chlorotic islands on infected leaves. Unlike leaf and stem rusts, stripe rust spreads consistently beyond the initial infection point (Roelfs *et al*., 1992). Each uredia may contain thousands of urediniospores (Fig. 2.1a). A single urediniospore is not visible with the naked eye but in masses appear yellow. The pathogen reduces plant vigor because it removes plant nutrients and water and results in desiccation of leaves (Chen, 2005). Later in the season black teliospores are formed on wheat leaves (Fig. 2.1c) (Knott, 1989).

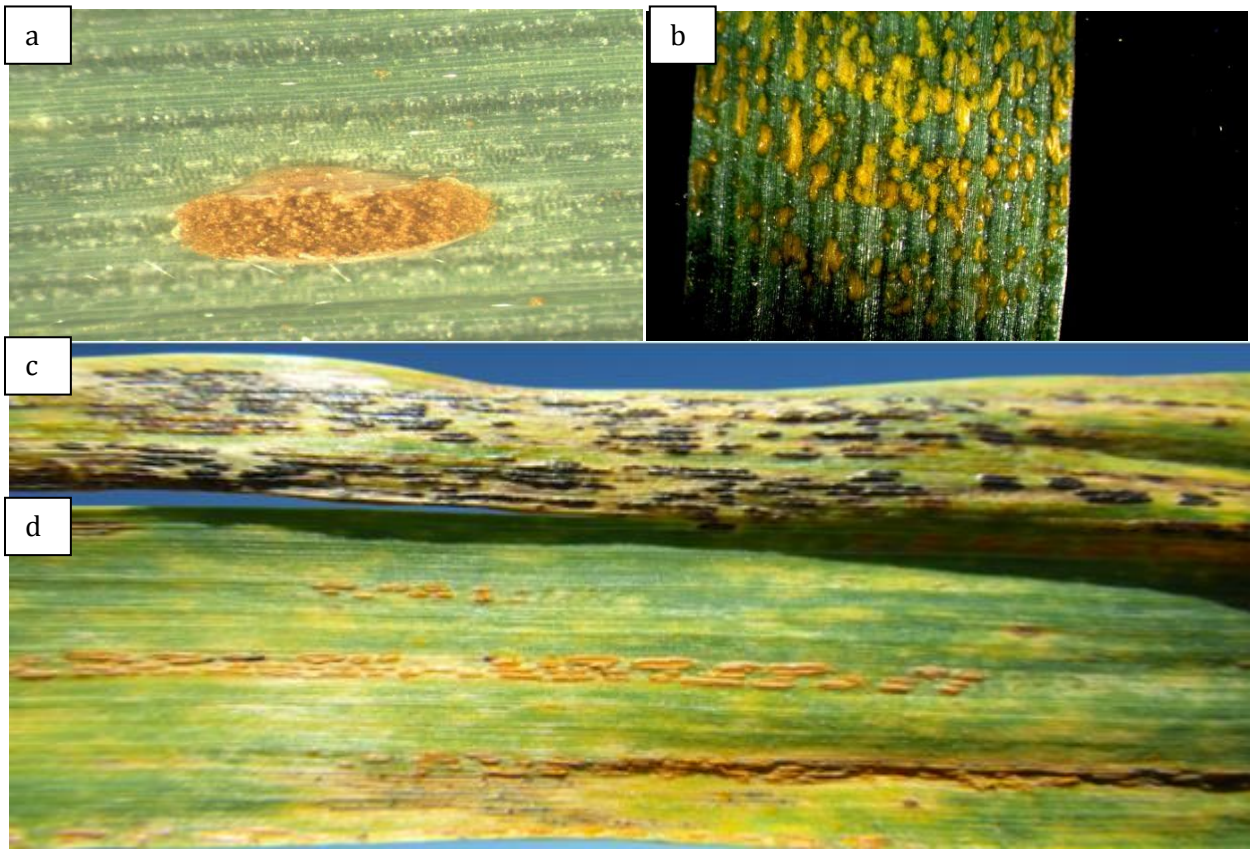


Figure 2.1. Symptoms produced by the fungus on the wheat plant: (a) microscopic view of a single pustule (sorus) on a susceptible seedling, (b) microscopic view of symptoms on a

seedling leaf, (c) black teliospores of *Puccinia striiformis* f. sp. *tritici* on adult plants produced later in the season, and (d) yellow urediniospores of the fungus with pustules in stripes along veins of the leaf.

2.2.3.2. Disease symptoms on alternate hosts

Germinating teliospores produce basidiospores, which are able to infect the alternate hosts of *Pst* (Jin *et al*., 2010; Zhao *et al*., 2013). The basidiospores infect barberry (*Berberis* spp.) leaves and produce sub-epidermal flask shaped pycnia on the upper surface and aecia on the lower surface. Pycnia contain pycniospores and aecia, aeciospores. Reddish pycnia and aecia are produced on Oregon grape (*Mahonia aquifolium*), which is closely related to barberry and identified as another alternate host of *Pst* (Wang and Chen, 2013).

2.2.4. Life cycle and infection process

The biology and epidemiology of *Pst* was studied extensively (Emge *et al*., 1975; Goddard, 1976; Joshi and Palmer, 1973; MacDonald and Strange, 1976; Shaner and Powelson, 1973; Shaner, 1969; Stubbs, 1967; and Zadoks, 1965) and the life cycles of most cereal rust fungi were determined except for that of *Pst,* which was not clearly established until 2010 (Jin *et al.*, 2010). The fungus was presumed to be macrocyclic and heteroecious because it shares many features with other cereal rusts, but this was unconfirmed until 2010 (Jin *et al*., 2010).

Unsuccessful attempts were made by researchers in the 1900s and 1930s to identify alternate hosts by inoculating them with germinating teliospores (Jin *et al*., 2010; Line, 2002). Production of diploid (2n) teliospores, formed by karyogamy, varies even under similar environmental conditions (Chen *et al*., 2012). Teliospores germinate at

approximately 12˚C in free water and produce a promycelia of four cells. Meiosis then produces a single haploid nucleus that later forms the basidiospore. Germinating basidispores infect barberry and produces pycnia containing pycniospores on the upper leaf surface (Chen *et al*., 2014). Pycniospores are of two mating type hyphae (+ and -) and after hybridization produce aecia and aeciospores on the lower leaf surface. Aeciospores infect wheat to produce uredeiniospores (Knott, 1989). Species in the genus *Berberis* are the hosts of a number of *Puccinia* spp. and were considered to be the host of *P. striiformis* as well by Mains (1933). However, Hart & Becker (1939 cited in Roelfs, 1967) failed to infect *Berberis* or *Mahonia*. These unsuccessful attempts led researchers to postulate that the alternate host did not exist and prolific asexual reproduction of the fungus through urediniospores, facilitated survival through re-infection/ secondary infection (Hassebrauk, 1970 cited in Line, 2002). Tillers and wild grasses were suggested to play a role in the oversummering of the pathogen as urediniospores, especially in the mountains (Hassan, 1968; Joshi *et al*., 1976; Joshi and Palmer, 1973; and Zadoks, 1961).

Rapilly (1979) hypothesized that the alternate host of *Puccinia striiformis,* may escape infection because of the short dormancy of the teliospores and the rapid production of basidiospores (Wright and Lennard, 1978). The complete life cycle of *Puccinia striiformis* was not known until 2010, when an alternate host was identified (Jin *et al*., 2010), thus contributing to a clear understanding of its phylogenetic relationship with other cereal rusts. Aeciospores of *Pst* were observed on *Berberis chinensis*, *B. koreana* and on 'Emerald Carousel' (an interspecific hybrid of *B. koreana* and *B. thunbergii*) in 2009; however, *Puccinia graminis*, the rust pathogen of *Berberis* in North America was unable to infect these *Berberis* species. Genetic analysis concluded *P. striiformis* f. sp. *poae* to be the cause of

these aecial infections. This was a starting point to further investigate the life cycle of *Pst*. To prove *Berberis* spp. were the alternate hosts of *Pst*, they were inoculated with germinating teliospores from infected wheat leaves. The aeciospores produced on *Berberis* spp. were inoculated on wheat to prove *Berberis* spp. were alternate hosts of *Pst* (where sexual reproduction occurs). The life cycle of *Pst* consists of pycniospores (*Berberis*)- aeciospores (*Berberis*)- urediniospores (wheat)- teliospores (wheat). Basidiospores are produced from teliospores very quickly and have little known function in the life cycle of *Pst* (Jin *et al*., 2010). After discovery of the alternate hosts of *Pst*, several other *Berberis* species were identified as alternate hosts in China (Zhao *et al*., 2013).

Uredinia are produced in stripes on the wheat leaf surface. Spores are produced in these pustules until the leaf senescences, and the teliospores form around the edges of the pustules. Basidiospores are produced from germinating dikaryotic teliospores after the crop season, which are able to infect *Berberis* spp. and thus produce basidiospores. Basidiospores produce pycnia on upper surface and aecia on lower surface of the barberry leaf. Aeciospores infect wheat and produces uredinia, containing urediniospores (Jin *et al*., 2010). Upon germination of urediniospores, germ tubes develop that penetrate the stoma, where substomatal vesicles are formed (Moldenhauer *et al*., 2006). Haustorial mother cells are formed from 2-3 infection hyphae that arise from the substomatal vesicle. Haustoria are formed in plant cells by invaginating the plasma membrane (Kang *et al*., 2002; Ma and Shang, 2009; and Mares, 1979); the haustoria nourish the fungus by drawing water and nutrients from the host plant (Voegele *et al*., 2009). Most of each haustorium (~85%) is located in leaf mesophyll cells, but part (~15%) is present in the leaf epidermal layer (Hovmøller *et al*., 2011).

2.2.5. Center of origin, epidemiology and dispersal

Early studies by Hassebrauk and Leppik in 1965 suggested the Transcaucasia (Fig. 2.2) as the center of origin of *Puccinia striiformis,* where wild grasses harbor stripe rust and the pathogen spreads from there in all directions (Hovmøller *et al*., 2011; Stubbs, 1985). Ali *et al*. (2010) considers Asia, and China in particularly, to be a center of origin of *Pst* based on prolific teliospore production by recombinant populations compared to clonal populations.

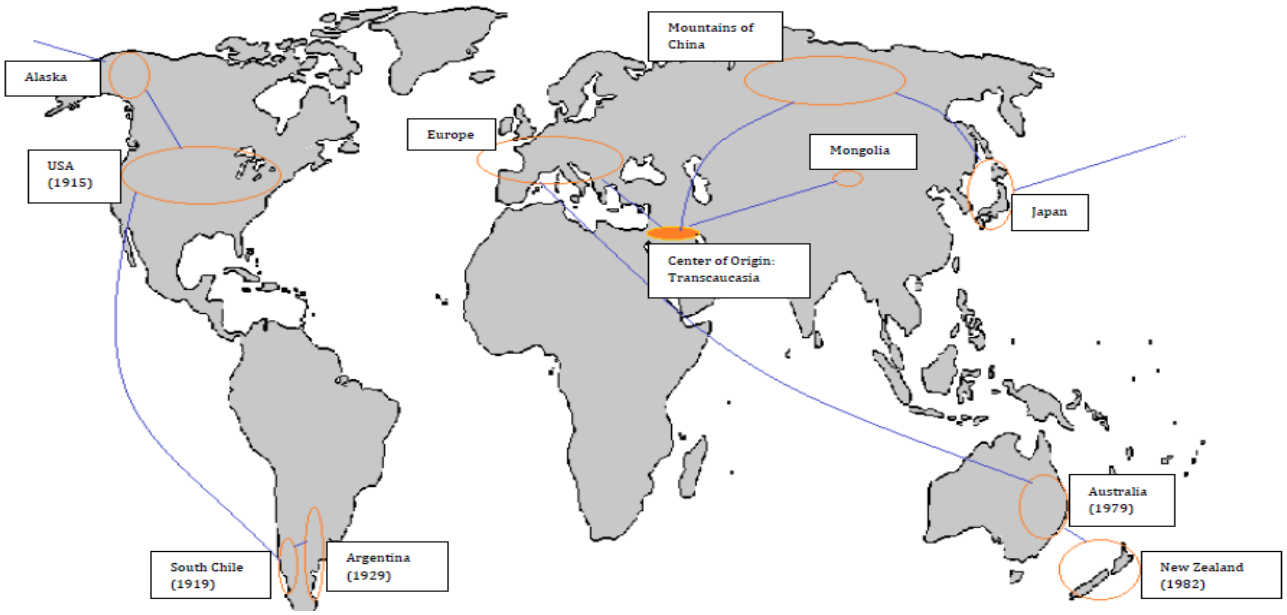


Figure 2.2. The dispersal path of *Puccinia striiformis* f. sp*. tritici* from the centre of origin (Transcaucasia) to other countries and continents (Ali *et al*., 2014; Stubbs, 1985; Wellings, 2007).

Urediniospores of *Pst* are dispersed by air over long distances (Chen, 2005) among regions or even between continents (Hovmøller *et al*., 2011). Urediniospore dispersal may also account for the expansion of the infected area in the same region (Hovmøller *et al*., 2011). The eastern pathway for dispersal of *Pst* is from the Transcaucasia to mountain

ranges of China and then to Japan (Stubbs, 1985). From Japan, *Pst* most likely entered the US via the Aleutians islands and Alaska. From there it spread south to Chile, from the western mountains of the US and to Argentina via Andean-Patogonian valleys. The northern spread of *Pst* is from the Transcaucasia to Mongolia. The pathogen spread southward along the western mountain ranges to South America from the center of origin (Stubbs, 1985). Stripe rust moved into Europe from the Near East, and from Europe it was introduced to Australia in 1979 and New Zealand in 1982 (Stubbs, 1985; Wellings, 2007). Stripe rust is believed to have evolved more recently than stem and leaf rust (Stubbs, 1985).

Stripe rust disease development is mainly dependent on three environmental factors: moisture, temperature and wind. Moisture is responsible for germination, infection and survival of rust spores. Urediniospores require moisture on the host surface for germination and infection. Irrigated wheat fields are more heavily attacked by *Pst* than dryland wheat because irrigation creates a favorable environment for disease development. Light showers in rainfed areas are also conducive to disease development. Rain and irrigated conditions create high moisture in soil and air, thus more dew formation occurs at night, which ultimately favors disease development. Rain is also helpful in spore dispersal. Urediniospores generally germinate shortly after production, provided enough moisture is present and temperature is optimum. Dry urediniospores can survive longer than wet urediniospores, so long distance dispersal of the spores is not an issue. High relative humidity facilitates contact between the urediniospores and the plant surface and also increases disease severity by triggering more urediniospores to germinate (Rapilly, 1979).

Temperature governs spore germination, the infection process, the latent period, sporulation, spore survival and host resistance. Cool weather favours stripe rust infection and disease development. The optimum temperature for the germination and infection process of *Pst* is 11^0C (minimum 0^0 and maximum 23^0) (Chen, 2005). Of the three wheat rusts, the optimum temperature is the coolest for *Pst* (Hogg *et al*., 1969). Experiments conducted by Milus *et al*. (2006) on the host-pathogen interaction in relation to temperature concluded that new races of the pathogen collected in 2000 or more recently are more aggressive and can flourish even at temperatures warmer than races collected prior to 2000. Night-time temperature is more important for stripe rust infection than daytime temperature (Stubbs, 1985). Conditions favorable to infection more often occur at night than during the day because favorable temperature and dew occur together. Temperature also affects survival of spores in winter (Chen, 2005). A temperature of -10^0C is detrimental to spore survival (Rapilly, 1979).

Wind may hamper spore germination and infectivity because it can dry urediniospores, although this also increases the duration of spore viability. The major role of wind is in pathogen dispersal from infected fields to healthy fields. Wind spreads the spores over long distances (Chen, 2005). It is suggested that primary inoculum originates at a site far from the actual site of infection, and wind plays an important role in long-distance dispersal of the urediniospores. The spread of races over long distances was reported (Hermansen and Stapel, 1973; Zadoks, 1961).

Light intensity was reported to have effects on the disease. Plants show a resistant reaction at high light intensities, but are susceptible at low light intensities (Stubbs, 1985).

It was demonstrated that *Pst* is more sensitive to air pollution than other cereal rusts (Sharp, 1967). Large ions of gases and other molecules in air decrease urediniospores germination (Sharp, 1967). Ultraviolet (UV) light is more detrimental to urediniospores of *Pst* as compared with spores of *Puccinia graminis* (Maddison and Manners, 1972).

Climatic and dispersal conditions that prevail at the time of spore formation affect spore germination (Gassner & Straib, 1934 cited in Stubbs, 1985; Sharp, 1965). Spores that are formed between 5-10^{0}C have the highest germination; those formed above 30^{0}C do not germinate (Stubbs, 1985; Line, 2002). Moderate frost has no adverse effect on urediniospore germination (Hassebrauk and Schroeder, 1965) and heat shock increases germination (MacDonald and Strange, 1976). The optimum temperature for urediniospore germination and appresoria formation is 7^{0}C (maximum 15^{0}C and minimum 2^{0}C). Germination of urediniospores of the same isolate may vary (Rapilly, 1979) because of the *cis*-3,4-dimethoxycinnamate compounds present (Macko *et al*., 1977). Germination of urediniospores is always greater on the upper leaf surface than the lower surface (Stubbs and Plotnikova, 1972).

2.2.6. Host-pathogen interaction

The host-pathogen interaction in wheat-*Pst* is governed by the gene-for-gene theory (Flor, 1947; 1955). *Puccinia striiformis* may be virulent or avirulent based on its capacity to cause disease and the host resistant or susceptible based on reaction to attack by the pathogen. The interaction of host and pathogen results in an infection type (IT), that can be scored as low or high (Knott, 1989). Resistance genes in the host are independent of each other and if there is more than one resistance gene in the host, the gene responsible for the low

infection type is expressed and the expression of other genes masked (Flor, 1955). This has been called 'false epistasis' (Knott, 1989). Interactions between resistance genes in the host are not common. Avirulence genes in the pathogen are reported to act independently, but some exceptions are reported (Knott, 1989). Host-pathogen interactions can be divided into specific and non-specific interactions. Specific interactions are the basis of the gene-for-gene theory (Flor, 1947, 1955). In race-specific interaction, there is an interaction between the genotype of the host and the genotype of the pathogen (Flor, 1947). A similar reaction of one host genotype to different pathogen isolates are non-specific interactions (Roelfs *et al*., 1992). Vanderplank (1963) used the terms 'vertical resistance' and 'horizontal resistance' for 'specific' and 'non-specific' resistance, respectively.

Biffen (1905) showed for the first time that resistance to a plant pathogen could be conditioned by a single gene. Following Biffen, many scientists performed experiments on wheat resistance to stripe rust and it was Knott (1989) who reported that resistance to stripe rust was often attributed to one or a few genes.

The symbol '*Yr'* is used to designate specific resistance gene against stripe rust (Lupton and Macer, 1962). Resistance to stripe rust is described as 'seedling resistance' and 'adult plant resistance (APR)'. Fifty-five *Yr* genes (41 seedling resistance genes and 14 adult plant resistance genes) have been identified to date (Chen, 2014). Seedling resistance is presumed to be conditioned by a single gene; thus it usually is race-specific. Resistance developed by plants subsequent to the seedling stage could also be race-specific (Knott, 1989; Zadoks, 1961). Genes *Yr1* to *Yr14* are known to be race-specific (Knott, 1989). Seedling resistance is effective throughout a plant's life, but unfortunately, *Pst* develops

new races very rapidly and has rendered single-gene resistance short-lived except resistance conditioned by APR genes (Yahyaoui *et al.*, 2002). Adult plant resistance develops as plants mature and is considered more durable than seedling resistance (Chen and Line, 1995). Wheat cultivars showing APR are often susceptible at the seedling stage, but show moderate to high resistance at the adult stage (Li *et al*., 2006). Adequate resistance is not provided by APR genes in wheat cultivars, but impart high resistance when combined with 4 or 5 minor genes (Singh *et al*., 2012). Adult plant resistance becomes effective at the tillering stage, gradually increases as the plant matures and is most commonly recognized at the booting stage. As the plant matures, haustorium formation is affected, differentiation of secondary intercellular hyphae and development of micro colonies of fungal hypahe is inhibited, especially at the boot stage (Zhang *et al*., 2012). Resistance in the host can be categorized based on a number of parameters that may be similar to each other in one way or other (Table 2.2).

Selected Canadian spring wheat cultivars were evaluated for resistance by planting in a stripe rust nursery (McCallum *et al*., 2007b). Screening and breeding for stripe rust resistance has been done in soft white spring (SWS) as these varieties were prone to attack by stripe rust in southern Alberta.None of the cultivars showed a high level of resistance, but cultivars carrying *Lr34/Yr18* show partial resistance. The *Lr34* gene for leaf rust resistance is completely linked to the *Yr18* gene (an APR gene for stripe rust), and it is likely that cultivars showing partial resistance may carry *Yr18* (McCallum *et al*., 2007b). Most wheat varieties grown in western Canada are susceptible to stripe rust, but many have an intermediate level of resistance and some are resistant. Most varieties with intermediate to moderate resistance were reported to carry the *Yr18* gene (McCallum *et al*.,

2007). The *Lr46/Yr29* and *Lr67/Yr46* genes are reported to confer APR, similar to the *Lr34/Yr18* locus, and provide resistance to both leaf and stripe rust, which is popular with wheat breeders (Hiebert *et al*., 2010). Severe stripe rust was recorded throughout southern Alberta and Saskatchewan in 2011 and it was found that the winter wheat variety 'Radiant' (carrying *Yr10*) and the spring wheat variety 'AC Barrie' were very susceptible, whereas the soft white spring wheat variety AC Andrew and the durum variety Avonlea were intermediate in resistance and Lillian highly resistant (Kutcher *et al*., 2012).

Table 2.2. Types of resistance to *Puccinia striiformis* f. sp. *tritici* races in wheat[1, 2].

Resistance type	Description	Durability
Seedling/all-stage	Detectable at the seedling stage and remains effective throughout lifetime of the plant	Usually not durable
Adult-plant resistance (APR)	Can't be detected at seedling stage and is expressed only in adult plants	Usually durable
Race-specific	Specific to some races but not to others	Usually not durable
Complete/immune	No visible symptoms	Usually not durable
Partial/incomplete	Visible symptoms of low or high IT	Usually durable
Hypersensitive resistance (HR)	Necrotic lesions on infected portions of the plant	Usually not durable
Slow-rusting/non-HR	Susceptible IT but reduced aggressiveness and severity, i.e. rust develops slowly	…….
Qualitative/monogenic	Controlled by single gene and shows two distinct classes in a segregating population	Usually not durable
Quantitative/polygenic	Controlled by more than one gene and shows variation in a segregating population	Usually durable

[1]Chen, 2013; [2]Knott, 1989.

2.2.7. Disease management

Early seeding, foliar fungicide application and cultivation of resistant varieties are the main strategies to control wheat rusts (McCallum *et al*., 2007a). Cultivation of resistant

varieties of wheat against stripe rust is an effective, relatively inexpensive and environment friendly method of disease control (Kumar *et al*., 2012; Line and Chen, 1995; Robbelen and Sharp, 1978). Cultivars with race-specific genes usually remain effective for only a few years because the pathogen changes to virulent on individual resistant genes (Line and Qayoum, 1992; Line and Chen, 1995, 1996). Some plants are resistant to stripe rust at the adult plant stage (boot stage); such plants have high temperature adult plant (HTAP) resistance. These plants express some level of resistance to stripe rust near maturity and the flag leaf contributes greatly to grain filling (Chen, 2005). Combining HTAP with seedling-resistance and cultivating multiline varieties is suggested to be a good approach for stripe rust management (Chen, 2005). Planting a mixture of wheat varieties can decrease disease severity and may also reduce infection by more than one race on a single leaf (Li *et al*., 2012).

Marker assisted resistance gene identification is very effective (Chen, 2005). Molecular markers were exploited to transfer the *Yr5* and *Yr15* genes in the development of varieties resistant to almost all races of *Pst* (Chen, 2005). Stripe rust resistance was incorporated into the minor soft white spring wheat class in Canada by *Yr5* and *Yr15* (McCallum *et al*., 2007a).

Most Canadian wheat cultivars remained unscreened for stripe rust as it was not a serious problem in commercial wheat cultivation (McCallum *et al*., 2007a) until recently, as it was a problem only in irrigated wheat crops in southern Alberta (Conner *et al*., 1988). In Canada, use of fungicides for disease management is employed by farmers (McCallum *et al*., 2007a). Propiconazole (Tilt®) and tebuconazole (Folicur®) are two of the most common

fungicides used in Canada to control rust diseases of wheat (McCallum *et al*., 2007a). Quarantine is not helpful in the case of stripe rust because it is an air-borne disease (Roelfs *et al*., 1992).

2.3. Variation in the *Pst* population

Gene and genotype diversity are found in populations of *Pst*. A feature of gene diversity is virulence, which is defined as a qualitative trait describing a pathogen that is able to infect a host plant and reproduce on it (Vanderplank, 1963). The degree or measure of pathogenicity is the relative capacity to cause disease (the American Phytopathological Society, 2014) and genotype diversity is the differentiation among races based on a virulent pathogen. Diversity among isolates of *Puccinia striiformis* collected from different geographic regions will be greater than diversity among isolates collected from different hosts. Different factors are responsible for variation in pathogen populations. Mutation, somatic recombination and sexual recombination are listed as factors causing variation in *Pst* populations (Hovmøller *et al*. 2011). The sexual cycle, the parasexual cycle and somatic recombination all affect genotype diversity of *Pst*. In addition, in small populations, selection, migration and random genetic drift are also responsible for genetic variation.

Genetic variation caused by mutation results in the formation of new alleles and genotypes (Hovmøller *et al*., 2011). Genes for avirulence in the pathogen are generally recessive and mutations in recessive genes are much more common than in dominant genes (Knott, 1989). If a variety with a qualitative resistance gene is surrounded by a susceptible variety, the virulent mutants may increase in rust and extreme selection pressure will help to increase the number of mutants in the population that may give rise

to a new biotype or race (Knott, 1989). The role of mutation in the evolution of races of *Pst* was first explained by Gassner & Starib in 1932 (cited in Hovmøller *et al*., 2011) and their estimation for mutation frequency was 1.6/100,000-200,000 urediniospores (Stubbs, 1985). Straib, in 1937, was the first to suggest the theory of stepwise mutation in *Pst* (Stubbs, 1985). Oort, in 1955, supported this view and grouped races in a series; each series included the races developed from the same parental race (Hovmøller *et al*., 2011).

Changes in virulence of the pathogen were observed by Johnson *et al*. (1978), Kajiwara *et al*. (1968) and Stubbs (1968), when they induced mutations in the pathogen using different mutagens. The emergence of pathogenic races and very small changes in virulence in the pathogen may be due to the low frequency of mutation, which is ultimately because of small population size, lack of a selective advantage of new mutants of *Pst*, or spontaneous loss of mutants from the population because of small population size (Hovmøller *et al*., 2011). Mutation to virulence in the pathogen for *Yr9* in varieties grown near the Red Sea Region (Ethopia, Yemen, Eritrea) in 1987 caused a wheat rust pandemic (Louwers *et al.,* 1992). It was reported that the generation of new races is largely governed by stepwise mutation, where single avirulence genes are gained or lost to give rise to new races of *Pst* (Wellings and McIntosh, 1990; Hovmøller *et al*., 2002) similar to *Puccinia triticina* (Ordoñez and Kolmer, 2009). Stripe rust races since 2000 in the USA can be explained by the stepwise gain or loss of virulence (Chen *et al*., 2010). Back mutations, i.e. a change from virulence/recessive to avirulence/dominant was suggested to be unlikely in rust populations (Knott, 1989); however, this was reported for *Yr9* and *Yr32* (Hovmøller and Justesen, 2009) and *YrA* (Wellings and McIntosh, 1990).

The recent discovery of the alternate host of *Pst* may possibly explain variation in the pathogen as a result of sexual recombination (Jin *et al*., 2010). A multi-virulent race of *Pst* detected in the UK, France, Germany, Denmark and Sweden, produces abundant telia under field and green house conditions suggesting that this new race might be the result of sexual recombination (Hovmøller *et al*., 2011).

Somatic hybridization and selection are two other mechanisms of variation in *Pst*. Little and Manners (1969) were the first to demonstrate the role of somatic recombination in the development of new physiological races. The re-assortment of whole heterokayotic nuclei gave rise to two new races and the chances of somatic recombination were showed to be 10% by Little and Manners (1969). Growing cultivars with a single specific resistance gene to *Pst* induces strong selection pressure on the pathogen population and results in variation and population dynamics (Little and Manners, 1969).

2.4. Race structure studies of *Pst*

Race characterization has been an important strategy in disease management programs because the information provides an understanding of pathogen virulence and the risk of epidemics to wheat breeders and agricultural advisors (Hovmøller *et al*., 2011). The virulence patterns of current races must be known (Su *et al*., 2003).

Puccinia striiformis f. sp. *tritici* is a specialized pathogen in terms of the host-pathogen relationship and isolates can be divided into races on the basis of their reaction on wheat cultivars or genotypes (Chen, 2005). A new virulent race is able to overcome a previously effective disease resistance gene and can prevail over a whole region in a short period of time (Line and Qayoum, 1992). Rapid shifts in the pathogen's virulence pattern and

aggressiveness (measured by the ability to produce more spores per pustule and/or reduced latent period) make it necessary for researchers to conduct timely surveys of pathogen populations (Hovmøller *et al*., 2012).

Races are identified using a set of plant genotypes or near-isogenic lines, called differentials. Reaction patterns on differentials are assessed to determine the race structure of the pathogen. Assignment of an isolate to a race is based on reactions of the isolate on a differential set of wheat and barley genotypes (Line, 2002). A physiological race is defined as a genetically distinct group within a species of plant pathogen characterized by specificity to one or more cultivars of the host plant(s) (the Canadian Phytopathological Society, 2014). Hungerford & Owens (1923) first identified different races of *Pst* using wheat and some grasses, but it was Allison and Isenbeck who reported races in 1930 using only wheat cultivars (Chen, 2005). Race structure studies in *Pst* are conducted using near-isogenic lines and/or supplemental wheat lines (Knott, 1989). Habgood in 1970 proposed a decanary system to designate races of plant pathogens and Johnson used it for stripe rust in 1972 (Knott, 1989).

Virulence/avirulence tests: The designation of 'virulent' or 'avirulent' is assigned to the pathogen's responses on host cultivars or genotypes (Sharma-Poudyal *et al*., 2013) based on compatible or incompatible reactions respectively (Roelfs *et al*., 1992), plus the ability to reproduce (Vanderplank, 1963). The designation of 'virulent' or 'avirulent' should be based on observed deviation from the expected low infection type of any particular host gene rather than the deviation from nominal values set within the scale (McIntosh *et al*., 1995).

Worldwide Virulence Surveys: A total of 235 isolates of *Pst* were collected from different countries for race studies (Sharma-Poudyal *et al*., 2013). A total of 129 and 169 races of the pathogen were described using 20 single Yr-gene lines and 20 wheat differential genotypes, respectively that are used in the USA for race differentiation. In all countries, virulence to *YrA*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr25*, *YrUkn*, *Yr27*, *Yr28*, *Yr31*, *YrExp2*, Lehmi (*Yr21*), Paha (*YrPa1*, *YrPa2*, *YrPa3*), Druchamp (*Yr3a*, *YrD*, *YrDru*), Produra (*YrPr1*, *YrPr2*), Stephens (*Yr3a*, *YrS*, *YrSte*), Lee (*Yr7*, *Yr22*, *Yr23*), Fielder (*Yr6*, *Yr20*), Tyee (*YrTye*), Tres (*YrTr1*, *YrTr2*), Express (*YrExp1*, *YrExp2*), Clement (*Yr9*, *YrCle*) and Compair (*Yr8*, *Yr19*) was detected. None of the isolates were virulent on *Yr5* or *Yr15*. Virulence on *Yr10*, *Yr24*, *Yr32*, *YrSP* and Moro (*Yr10*, *YrMor*) was infrequent among isolates from all countries. Isolates from Canada were not virulent on *Yr5*, *Yr15*, *Yr24*, *Yr32* and *YrSP* (Sharma-Poudyal *et al*., 2013).

Race structure studies on Pst in Europe and the Middle East: It was Gassner and Straib in Germany who conducted experiments to determine the race structure of *Pst* in 1932 (Zadoks, 1961). They used different genotypes of wheat, barley and rye in their experiments. Race structure analysis was further examined in 1956 when the Netherlands faced a severe epidemic of the disease in 1955 (Zadoks, 1961). Fuchs in 1960, and Zadoks in 1961 used the same set of differential lines as Gassner & Straib (Chen, 2005) for race structure analysis in Europe. Later, Stubbs (1985) used this same set for an international race survey. Severe epidemics on a popular cultivar of wheat in the United Kingdom led to national race surveys in 1967 (Johnson, 1992). Rust resistance in a number of wheat and triticale cultivars in Europe has been overcome by newly evolved races (Chen, 2005). From 1997 to 2008, 14 pathotypes of the pathogen were detected in Denmark. The most

common pathotype detected in 2007 in Denmark was virulent on cultivars with *Yr17* and *Yr32*. The same pathotype was present in France at low frequency since 2000, but became common in recent years (Hovmøller and Henriksen, 2008).

In Turkey in 2009-2011, experiments concluded that *Pst* is virulent on *Yr2*, *Yr6*, *Yr7*, *Yr9*, *Yr18*, *Yr27*, *Yr28*, *Yr31* and avirulent on *Yr1*, *Yr3*, *Yr4*, *Yr5*, *Yr8*, *Yr10*, *Yr15*, *Yr17*, *YrSP*, *YrCV* (Tekdal *et al*., 2012). Again in Turkey, 54 isolates were differentiated into 27 and 45 races using 20 world and 20 US wheat differentials, respectively. None of the isolates were virulent on *Yr5*, *Yr10*, *Yr15* or Moro, which carries *Yr10* and *YrMor* (Sharma-Poudyal *et al*., 2013).

Race Studies in China: A set of wheat differentials different from Europe was used in China to determine the race structure of *Pst*. Nine races designated C1-C9 using seven wheat genotypes and one barley were identified in 1944 by Fang (cited in Line, 2002). Lu *et al*. (1956 cited in Chen, 2005) reported 16 races using Gassner & Straib's 14 differentials from 50 isolates (Chen, 2005). Later, it was found by Chinese scientists that the European set of differentials was not suitable for race structure studies in China and they grouped seven wheat genotypes in a differential set called Chinese yellow rust (CYR) and used numbers to name races of *Pst* (Wang *et al*., 1963). A total of 67 races were identified using 17 differential genotypes (Wan *et al*., 2004). In 2007, 41 races of *Pst* in China were identified using 19 differential lines from isolates collected between 2003 and 2007. A significant change in virulence pattern was observed along with great diversity (Chen *et al*., 2009). Virulence to *Yr5*, *Yr15*, *Yr24* and Moro (*Yr10*, *YrMor*) was not detected among the 60 isolates characterized using 20 US and 20 world differentials (Sharma-Poudyal *et al*.,

2013). Chinese races CYR32 and CYR33 have become the most important of the *Pst* races in recent years because of greater fitness on commercial cultivars (Chen *et al*., 2014).

Australian studies on race structure: In Australia, 15 races of *Pst* were reported in 10 years (Wellings and McIntosh, 1990). The first original race overcame *Yr2* and later, races were found to be virulent (in various combinations) on *Yr1, Yr2, YrA, Yr5, Yr6, Yr7, Yr8* and *YrSP*. In 2002, a new race of *Pst*, virulent on *Yr6, Yr7, Yr8, Yr9* and *YrA,* was detected in Western Australia, which spread to eastern Australia, indicating that it was a foreign introduction (Wellings *et al*., 2003; Wellings and Kandel, 2004). Five isolates were characterized as avirulent on *Yr5*, *Yr10*, *Yr15*, *Yr27*, *Yr32*, *YrSP*, Moro (*Yr10*, *YrMor*) and Yamhill (*Yr2*, *Yr4a*, *YrYam*) using 20 US and 20 world differentials (Sharma-Poudyal *et al*., 2013).

Race studies on the Indian sub-continent: Stripe rust is a major production problem of wheat in the Indian sub-continent. The disease is considered endemic to the north-western plains zone (NWPZ) (Prashar *et al*., 2007). Mehta (1933) started working on race studies of *Pst* in India using Gassner and Straib's method. Until the 1960s, there was no, or negligible, changes in the race structure of the pathogen (Mehta, 1933). A new race was observed to be virulent on the wheat variety 'Kalyansona' (*Yr2*), and it was considered to be one of the three races presumably introduced from the Near East (Sharma *et al*., 1972). Fourteen pathotypes of *Pst* were identified during 1994-95 and a few more in 2004; however, only four pathotypes were common in India (Prashar *et al*., 2007). The genes *Yr5*, *Yr10*, *Yr15*, *Yr32* and *YrSP* were found to be effective against all races and low virulence frequency was detected for genes *Yr2, YrYam*, *Yr4a* (in Yamhill), *Yr17*, *Yr24* and *Yr25* in Pakistan (Bux *et*

al., 2012). High virulence frequency and pathotype diversity was found in the Himalayan region of Pakistan. A total of 53 pathotypes were detected from 127 isolates and all were avirulent on the genes *Yr3*, *Yr10*, *Yr17*, *Yr26*, *Yr32* and *YrSP*. Virulence on *Yr5*, *Yr15* and *Yr24* was rare (Ali *et al*., 2014).

From Nepal, 20 and 15 races were detected using 20 US and 20 world differentials, respectively and all were avirulent on *Yr5*, *Yr15*, *Yr32*, *YrSP* (Sharma-Poudyal *et al*., 2013).

North American races of Pst: No research on race identification of *Pst* was carried out before 1960 in states located east of the Rocky Mountains in the USA because stripe rust was not observed as an economic disease (Chen, 2005). In the USA, Bever (1934) identified two physiological races of *Pst* in 1933, one race from Montana and another from Idaho. Epidemics of 1973 and 1974 in the PNW and California brought major changes in races (Line, 2002). The system of race structure identification in the US used at that time was developed by Line and his colleagues in 1968 (Line *et al*., 1970). Later, the number of differential lines used in race identification was increased to 20. In total, 109 races of *Pst* in the US were identified up to 2004, of which 59 were identified before 2000 and 50 after 2000.

As many races developed in 4 years (2000-2004), as were observed in the previous 40 years (before 2000) because the disease had become widespread and a greater number of differentials were used in recent years (Chen, 2005). Races were first described using the prefix CDL (after Cereal Disease Laboratory of USDA) but now PST (after the latin name of the fungus: *Puccinia striiformis* f. sp*. tritici*) is used as a prefix (Chen *et al*., 1995; Chen and Moore, 2002).

Seven epidemic regions in North America are described by Line and Qayoum (1992) on the basis of geographic features such as barriers, wind conditions, crop cycles, occurrence of rust, and *Pst* virulence on cultivars. Races of *Pst* differed greatly in these regions before and after 2000. Eastern Washington, northeastern Oregon, and northern Idaho of the USA, and southeastern British Columbia, Canada is included in Region 1, where climate favors pathogen overwintering and disease development on spring wheat. Cultivation of both winter and spring wheat is even more favorable for pathogen survival because of the continuous supply of a green host/green bridge. Before 2002, there was a great diversity of *Pst* races in Region 1 (Chen *et al*., 2003).

Western Montana, USA, and southern Alberta, Canada are included in Region 2. Southeastern Oregon, northern Nevada, northern Utah, southern Idaho and western Colorado, USA are part of Region 3. These two regions are not prone to epidemics of stripe rust because winters are very harsh and pathogen survival is negligible. All races prevalent in Region 2 and 3 were first observed in Region 1. In the case of an epidemic in Region 1, late attack of the pathogen in Region 2 and 3 results in greater risk of an epidemic in spring wheat in Alberta (Line and Qayoum, 1992). In Region 4 (northern California and western Oregon) wheat is attacked by local *Pst* inoculum and infection from other regions is rare (Line and Qayoum, 1992). However, races in this region are similar to races found in other regions (Chen and Moore, 2002; Chen *et al*., 2003, 2004). Stripe rust is frequently severe in Region 5, which is western Washington and southwestern British Columbia because of the favorable climate. It is a major center of diversity of *Pst* because the pathogen has existed in this region for more than 100 years (Humphrey *et al*., 1924). Other weed and accessory hosts of the pathogen may also contribute to its race diversity in North America. Region 6

(central California) is able to affect other regions (Mexico and Region 7 in particular) and can also be affected by pathogen populations from other regions, especially Mexico (Chen and Moore, 2002). Region 7 includes areas east of the Rocky Mountains from Texas, US, to Ontario, Canada, and from the eastern slopes of the Rocky Mountains in Colorado to Virginia, USA. Races found in this region in recent years are diverse and are the same as the races found in other regions. Races detected in the period 2000-2003 were prevalent in California and also in Region 7 (Chen and Moore, 2002; Chen *et al*., 2004).

Races prevalent in North America in recent years have a wide virulence spectrum. This is against the concept that isolates with fewer virulence genes are more aggressive (Vanderplank, 1963; Line and Qayoum, 1992). Race frequency is determined by virulence and fitness cost. *Pst* is an obligate parasite and therefore must be virulent on the host to grow and sporulate. A race with more virulence genes can attack more cultivars, and thus helps to increases its frequency in the population (Vanderplank, 1963; Line and Qayoum, 1992). The same host variety over a large area can influence virulence of a race by selection pressure. If the host has few resistance genes, then races with corresponding virulence genes will be favored and these races will prevail. Alternatively, if the host has many resistance genes then more races are likely to be present on such a host population. These two forces are responsible for the current status of *Pst* races in the USA (Milus *et al.,* 2006). In the USA, Chen *et al*. (2010) identified 18 races of *Pst* (of which 5 were new) in 2006, and 30 races in 2007 (of which 16 were new) using 20 differential wheat cultivars. In 2008 & 2009, 33 and 26 races were detected, respectively in the USA and 18 of these were detected in both years (Wan and Chen, 2012). The present epidemiological evidence divides the US into 12 regions. Regions 1 to 6 consist of the states mentioned by Line and Qayoum (1992).

Region 7 consists of Texas, Louisiana, Arkansas, Oklahoma, and eastern New Mexico; Region 8 consists of Kansas, Nebraska, and eastern Colorado; Region 9, South Dakota, North Dakota, Minnesota, and eastern Montana; Region 10, Mississippi, Alabama, Florida, Georgia, South Dakota, North Dakota, Tennessee, and Kentucky; Region 11, Missouri, Illinois, Indiana, Iowa, Wisconsin, and Michigan; and Region 12, Virginia, West Virginia, Ohio, Maryland, Pennsylvania, and New York (Sharma-Poudyal *et al*., 2014).

Stripe rust in Canada is affected by its occurrence and severity in the USA (Chen, 2005; Holtz *et al*., 2013; Kumar *et al*., 2012; Su *et al*., 2003). Regions 1, 2 and 5 also include the western Canadian provinces. Region 7 includes the eastern Canadian provinces (Line and Qayoum, 1992). Before 2000, stripe rust of wheat was only a problem on the western Canadian prairies, but after 2000 it also appeared in eastern Canada (Chen and Moore, 2002). British Columbia and south-western Alberta may provide a source of inoculum for most of western Canada where the pathogen may overwinter (Conner *et al*., 1988; Kumar *et al*., 2013).

Airborne inoculum from the PNW and the Great Plains of the USA also plays a role in infections in Canada (Sanford and Broadfoot, 1932). Two physiological races of *Pst* were reported by Newton *et al*. (1933) in Canada in 1932. Beginning in 2000, stripe rust was reported every year, and major epidemics in Saskatchewan and Central Alberta occurred in 2005 (McCallum *et al*., 2006) and 2011 (Kutcher *et al*., 2012). Su and co-workers (2003) reported 39 races of *Pst* in western Canada using 17 world and European differentials, plus 7 supplemental differentials from 57 isolates collected between 1984-2002 in western Canada. Before 2000, races virulent on two cultivars 'Lee' (*Yr7, Yr22*, *Yr23*) and 'Owens'

were observed only in British Columbia and races from the prairie provinces were avirulent on these two cultivars. New races detected in western Canada since 2000 are virulent on *Yr8*, *Yr9*, *Yr19*, and *YrCle* (Su *et al*., 2003). Some races observed after 2000 in the prairies were also found in the Creston area of British Columbia indicating possible linkage between inoculum dispersal. A race virulent on *Yr5* has not been identified. Genes *Yr1*, *Yr10, YrMor*, *Yr3b*, *Yr4b*, *Yr15* and *YrSP* are resistant to 90% of the races prevalent in western Canada, and all are resistant to new races detected since 2000 (Su *et al*., 2003).

In 2012, 13 pathotypes of *Pst* were identified from characterization of 38 isolates collected from central Alberta (Kumar *et al*., 2012). Wheat cultivars with genes *Yr1*, *Yr5*, *Yr15*, and *YrSP* were resistant to all 13 pathotypes and cultivars with genes *Yr10*, *Yr24* and *Yr28* were resistant to approximately 90% of the isolates. High virulence frequency of 71-100% was observed on *YrA*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr26*, *Yr27*, *Yr31* and *Yr32* (Kumar *e*t al., 2012). Later, 12 more pathotypes were detected from central Alberta (Holtz *et al*., 2013). Virulence on *Yr5* and *Yr15* was not detected and genes *Yr1*, *Yr10*, *Yr24*, *Yr28*, *YrTye* and *YrSP* were effective against more than 80% of the pathotypes. The most common pathotype, representing approximately 50% of the total *Pst* isolates characterized was avirulent on *Yr1*, *Yr5*, *Yr10*, *Yr15*, *Yr24*, *Yr28*, *YrSP*, Hyak (*Yr17*, *YrTye*), Moro (*Yr10*, *YrMor*), Paha (*YrPa1*, *YrPa2*, *YrPa3*) and *YrTye* (Holtz *et al*., 2013).

To transfer effective stripe rust resistance into newly developed cultivars, the virulence spectra (race structure) of the current pathogen population must be known (Su *et al*., 2003). Wheat crops have been damaged by cereal rusts for as long as wheat has been cultivated and plant breeders are in a continuous battle against increased virulence

frequency of new races. Resistance breeding has resulted in significant gains, but to maintain these gains constant monitoring of the rust pathogen is necessary (Hodson *et al*., 2012). Although the race structure of *Pst* was first conducted by Sanford and Broadfoot in 1932 in western Canada, then by Su *et al*. (2003) and recently by Kumar *et al.* (2012) and Holtz *et al*. (2013) in central Alberta, detailed knowledge of *Pst* in Canada is lacking, especially in Saskatchewan, where no race structure studies of *Pst* have been conducted. Moreover, recent epidemics of stripe rust on the prairies makes it imperative that we understand the virulence patterns of *Pst* in Saskatchewan and the Canadian prairies.

2.5. SNP polymorphism and diversity analyses

Allelic variations within a genome of the same species can be classified into three main groups: repeated sequences of a very few base pairs of DNA [microsatellites, or simple sequence repeats (SSRs) or short tandem repeats (STRs)], insertions or deletions of bases in the DNA (InDels), and single nucleotide polymorphisms (SNPs) (Mammadov *et al*., 2012). Genetic tools to detect these variations in the DNA are called molecular or DNA markers. Although SSRs, InDels and SNPs are the three main types of allelic variations in any individual or progeny, there is a wide range of molecular markers available to detect polymorphisms in DNA sequences (Gupta *et al*., 1999). Low coverage of sequence data (i.e. <5X), make accurate SNP calling and genotype calling difficult and results thus obtained may not be reliable (Nielsen *et al*., 2011).

Single nucleotide polymorphisms are a relatively new class of molecular marker and SNPs are found in abundance in the genome of any organism. Single nucleotide polymorphisms can be used to discover polymorphisms in coding and non-coding regions,

thus covering a large part of the genome (Morin *et al*., 2004). A SNP is a single base pair mutation at a specific locus and SNPs are conserved in the genome during evolution. Because SNPs are conserved during evolution, these markers are preferred over microsatellite markers for association studies (Harbron and Rapley, 2004). Variable null alleles and mutation patterns make microsatellite markers unsuitable for data analyses by introducing ambiguity. With SNP markers, mutations observed as single nucleotide polymorphic sites are very abundant and can be explained by simple mutation models. Using SNP loci in population studies is advantageous because SNPs represent the whole genome very well and there is less interlocus sample variance. The increased number of SNPs can help to identify loci under selection (represented by outliers). By sequencing the whole genome, the genomic regions under selection can be identified using SNP markers. In microsatellite markers, mutation rates are very high and homoplasy (the parallel evolution of identical character states) is common and thus yields unreliable decisions about gene flow among populations (Morin *et al*., 2004).

Various markers such as AFLP, RAPD (Becerra *et al*., 2007), and SSRs were employed to study genetic diversity in *Pst* of wheat (Holtz *et al*., 2013), and these studies suggest high variability among *Pst* populations. Diversity analyses for cereal rust populations using SNP markers is not common, but SNP markers are the most recent and have the potential to provide a better idea of population structure than other methods.

2.6. Next-generation sequencing (NGS) platforms

Technology for genome sequencing is emerging as a new tool and evolving rapidly. Several new platforms in this series of genome sequencing technology were added in recent years,

such as the Ion Torrent Personal Genome Machine and the Pacific BioSciences (PacBio) RS (Quail *et al*., 2012). Next generation sequencing (NGS) is a recent, promising and cheap technology (Nielsen *et al*., 2011; Ratan *et al*., 2010) to aid in the understanding of variation over the whole genome of an organism (Seeb *et al*., 2011), and it is based on the principle of sequencing-by-synthesis (Nielsen *et al*., 2011). NGS is a powerful tool that has helped in genome sequencing by increasing speed and depth, along with a steep decline in the cost compared with traditional sequencing techniques. Application of NGS to host-pathogen interactions should help plant pathologists because it shortens the time required to collect molecular information on the pathogen (Cantu *et al*., 2011). We can use NGS for identifying single nucleotide polymorphisms (SNPs) and sampling of a complex genome. NGS technologies can produce much data in a short period of time, even in non-model organisms (Fu and Peterson, 2011; Seeb *et al*., 2011). In NGS, 'SNP calling' identifies variable sites in the genomic region, and 'genotype calling' determines the genotype of every individual at each site (Nielsen et al., 2011).

Illumina has released several next generation sequencing platforms, such as illumina MiSeq, illumina HiSeq2000, illumina HiSeq 2500, etc. (www.illumina.com). The rate of correctly called SNPs for illumina platforms is 68-76%. Sequencing costs for illumina platforms is lower than for other platforms, such as PacBio RS, and Ion Torrent PGM. Within different Illumina platforms, the cost is lower for Illumina HiSeq compared to MiSeq and GAIIx. For the Illumina platforms, indel (insertion/deletions) errors during base calling are rare (Nielsen *et al*., 2011) but overall error rates are below 0.4% (Quail *et al*., 2012). Next-generation DNA sequencing platforms have the ability to detect polymorphisms on a

genome-wide scale but their utility in plant pathogen diversity analysis is not well understood (Cantu *et al*., 2011).

2.7. Summary

In conclusion, it is clear that *Pst* changes rapidly over a short period of time and the virulence spectrum of recently detected races is wider than the races detected in the past. Many races of *Pst* were detected in wheat growing countries in past 25 years and virulence surveys are conducted every year in rust affected areas. For successful management of the disease, regular monitoring of the pathogen population is required. Advanced molecular and sequencing techniques can be very helpful in better understanding the pathogen population.

CHAPTER 3. STRIPE RUST OF WINTER WHEAT, SPRING WHEAT AND BARLEY IN SASKATCHEWAN IN 2013 AND 2014

3.1. Preface

Surveillance of pathogens, including assessment of incidence, severity and geographical occurrence is important in driving national and international policies, plant breeding and pathology research for successful management of crop diseases. This is achieved by surveying commercial crops or trap plots at multiple locations. This chapter discusses the status of stripe rust of wheat and barley in Saskatchewan in 2013 and 2014, which forms the basis of virulence and genotypic characterization of *Pst* in western Canada.

3.2. Introduction

Stripe rust of wheat and barley, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) and *P. striiformis* f. sp. *hordei* (*Psh*), respectively are very common on commercial wheat and barley crops in North America (Chen, 2005). The distribution of stripe rust races on the Canadian prairies is governed by races prevalent in the USA (Chen, 2005; Su *et al.*, 2003). Races of *Pst* and *Psh* in western Canada are a result of air-borne inoculum of the pathogen from either the Pacific North-West (PNW) of the USA (Kumar *et al*., 2012; Su *et al*., 2003) or inoculum that has travelled along the 'Puccinia pathway'. Stripe rust in Manitoba and eastern Saskatchewan is largely due to the latter and the likelihood of inoculum arriving from the PNW is rare (Brent McCallum and Tom Fetch, personal communication). Stripe rust has appeared as a regular pest of wheat in western Canada after 2000 (McCallum *et al*., 2003, 2004). The overlapping host boundary of *Pst* and *Psh* is still a mystery (Chen, 2005). Widespread epidemics of stripe rust in western Canada in 2005 (McCallum *et al*., 2006),

2006 (McCallum *et al*., 2007c) and 2011 (Kutcher *et al*., 2012), highlighted the importance of this disease. For successful management, regular monitoring and disease surveys are required.

3.3. Materials and methods

Eighty-six commercial fields (late milk to soft dough stage) of winter wheat, spring wheat, barley and susceptible wheat lines in three trap plots of stripe rust differentials were assessed for disease symptoms of stripe rust (*Puccinia striiformis* f. sp*. tritici* and *P. striiformis* f. sp. *hordei*) in 12 crop districts of Saskatchewan between early July and early September, 2013. In 2014, 38 winter and spring wheat crops were surveyed from late July to late August in 12 crop districts of Saskatchewan. Four trap plots of wheat stripe rust differentials were planted in Saskatchewan at Scott, Swift Current, Prince Albert and Saskatoon. The crops surveyed were separated from each other by at least 20 km. Each field was traversed in a "V" pattern (Puchalski *et al.,* 2012) within which individual plants from five sites separated by about 40 m were evaluated for incidence and severity of stripe rust. Incidence was estimated as the proportion of infected plants exhibiting at least trace levels of stripe rust in a five m row at each site in the crop. The modified Cobb scale (Peterson *et al*., 1948) was used to assess stripe rust severity on the flag leaves of 50 plants per crop (ten leaves per site). A six-category scale was used to determine stripe rust severity in each field: clean (no visible symptoms); trace (<3% leaf area affected); light (3-5%); moderate (>5-20%); and severe (>20%).

3.4. Results and discussion

Generally, temperatures in Saskatchewan in 2013 were below normal for most of the summer, but somewhat above normal in late August and throughout September. There was limited precipitation in May and little in August or September, but frequent precipitation in June and July. Teliospore formation and senescence of plant tissue were observed by mid-August. In 2014, temperatures in Saskatchewan were above normal for most of the summer. Saskatchewan received good precipitation in July and August but it was not uniformly distributed. Precipitation was below normal for May, above normal for June and August and close to normal in June.

Many commercial winter wheat crops in Saskatchewan are sprayed with foliar fungicides and thus rust development was likely reduced or prevented in many of the fields surveyed. In 2013, stripe rust was observed in 26 winter wheat crops (30%), the three trap plots, 8 spring wheat crops (47%) and 2 barley crops (7%). Of the 86 commercial winter wheat crops, 60 (70%) were clean and three (3%) had trace levels of stripe rust. Eleven (13%) were rated as light, five (6%) as moderate and seven (8%) as severe (Table 3.1). Stripe rust-susceptible wheat genotypes and differentials, such as Avocet -*YrA, YrA, Yr6, Yr7, Yr8, Yr9* and AC Barrie (both spring and winter), in trap plots was rated as moderate at Swift Current and severe at Melfort and Scott. Crop Districts 6B and 9A (Figure 3.1; Table 3.1) had the highest and lowest levels of severity, respectively. Severe infection was observed on the winter wheat cultivar 'CDC Falcon' (fungicide unsprayed) at Insinger, SK (Crop District 5A). Only two barley crops (one each in Crop Districts 6B and 8B) of the 30 surveyed were affected by stripe rust. The incidence was 3% and severity was 5% on both crops.

In spring wheat, stripe rust was severe in one crop in crop district 8B and observed only at trace levels in all other crop districts (Table 3.2). The distribution of stripe rust in Saskatchewan in 2013 is showed in Figure 3.2.

Stripe rust was observed in eight wheat crops in 2014 (21%) and susceptible differentials (i.e. Avocet -*YrA* and Avocet *+YrA*) in the four trap plots: Saskatoon (central SK), Prince Alberta (northern SK), Scott (west-central SK) and Swift Current (southern SK). Of 38 wheat crops surveyed: 30 (79%) were clean and two (5%) had trace levels of stripe rust (Table 3.3). Two (5%) were rated as light, one (3%) as moderate and three (8%) as severe. Stripe rust was severe on Avocet -*YrA* (susceptible check) at Prince Albert and moderate at Saskatoon and Swift Current. Crop district 4B had the highest levels of stripe rust. The three crops with severe levels of stripe rust were observed in crop districts 4A, 4B and 3B-N. Severe stripe rust in 2013 in a few fields may have been on susceptible varieties lacking APR genes and not because of early disease on-set as disease was not observed anywhere in SK at the seedling stage.

Stripe rust was frequently observed and uniformly distributed across the province in 2013. In 2014, stripe rust was observed in 5 of the 12 crop districts surveyed (Table 3.3). Stripe rust incidence was low in 2014 in the USA as compared to 2013 (Anmin Wan, personal communication). This might be the reason for the low levels of stripe rust observed in Canada because the inoculum arrives from the USA. Stripe rust was observed in southern Alberta at moderate to severe levels in 2014 (Andre Laroche and Denis Gaudet, personal communication), which may be the reason for stripe rust occurrence in south-western SK. The low levels of stripe rust in 2014 are also evident from low levels of infection on susceptible differentials in the three trap plots in SK.

The hot, dry winds in southern SK may have prevented rust development. In crops where stripe rust was observed, teliospore formation was observed by late July and early August.

Stripe rust was not observed in 2014 in crop districts 5A, 9A-E, 9A-W, 8B, and 7A, which are in the northern and the eastern areas of SK. This further supports the incursion of stripe rust inoculum from southern Alberta into southern Saskatchewan.

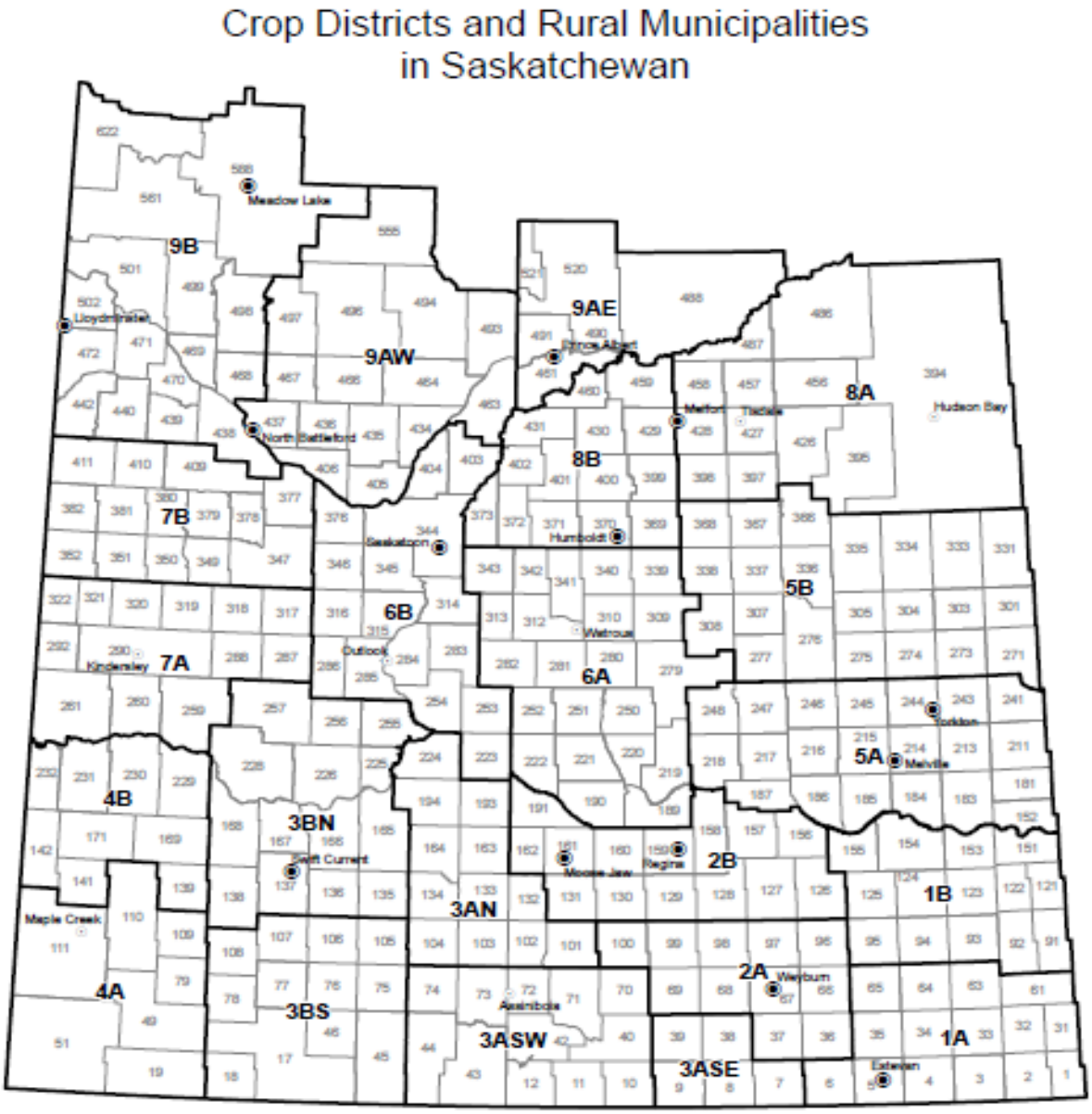


Figure 3.1. Map of Saskatchewan showing crop districts (adopted from: http://agriculture.gov.sk.ca)

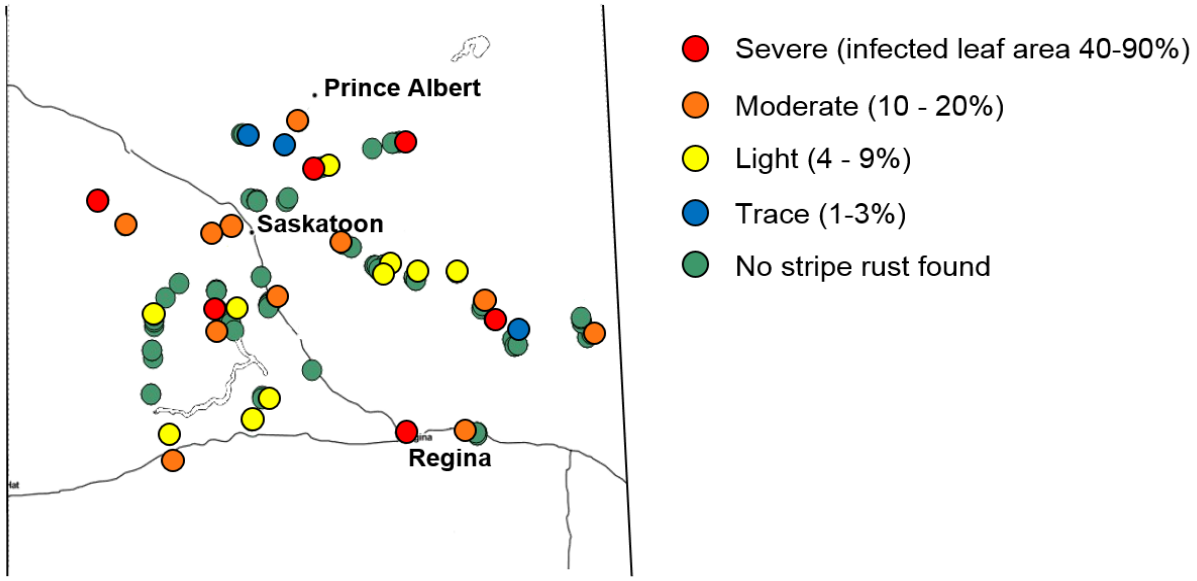


Figure 3.2. Stripe rust distribution in Saskatchewan in 2013.

Table 3.1. Prevalence (proportion of crops affected) and severity of stripe rust on commercial winter wheat crops in 2013 in Saskatchewan by crop district.

Crop District	Prevalence	Severity				
		Clean	Trace	Light	Moderate	Severe
2B	1/5	4	0	0	0	1
3A-N	2/4	2	0	2	0	0
3B-N	1/7	6	0	1	0	0
5A	4/9	5	1	0	2	1
5B	3/10	7	0	2	0	1
6A	3/11	8	0	2	1	0
6B	4/17	13	0	1	0	3
7A	1/4	3	0	1	0	0
7B	1/2	1	0	0	1	0
8A	0/5	5	0	0	0	0
8B	3/6	3	0	1	1	1
9A	3/6	3	2	1	0	0
Total	**26/89**	**60**	**3**	**11**	**5**	**7**

Table 3.2. Prevalence (proportion of crops affected) and severity of stripe rust on commercial spring wheat crops in 2013 in Saskatchewan by crop district.

Crop District	Prevalence	Severity				
		Clean	**Trace**	**Light**	**Moderate**	**Severe**
2B	0/4	4	0	0	0	0
3B-N	1/4	3	1	0	0	0
5A	1/1	0	1	0	0	0
5B	1/2	1	1	0	0	0
6A	2/3	1	2	0	0	0
8B	3/3	0	2	0	0	1
Total	**8/17**	**9**	**7**	**0**	**0**	**1**

Table 3.3. Prevalence (proportion of crops affected) and severity of stripe rust on commercial winter wheat crops in 2014 in Saskatchewan by crop district.

Crop District	Prevalence	Severity				
		Clean	Trace	Light	Moderate	Severe
3A-N	0/4	4	0	0	0	0
3B-N	1/9	8	0	0	0	1
4A	1/2	1	0	0	0	1
4B	4/4	0	1	1	1	1
5A	0/2	2	0	0	0	0
6B	1/4	3	1	0	0	0
7A	0/2	2	0	0	0	0
7B	0/3	3	0	0	0	0
8B	0/1	1	0	0	0	0
9A-E	0/1	1	0	0	0	0
9A-W	0/2	2	0	0	0	0
9B	1/4	3	0	1	0	0
Total	**8/38**	**30**	**2**	**2**	**1**	**3**

3.5. Conclusion

In conclusion, stripe rust is a common pest of wheat in Saskatchewan. Severe levels of stripe rust on susceptible varieties have the potential to cause significant yield losses. Stripe rust inoculum in SK is the result of wind blown spores either from southern AB or along the 'Puccinia Pathway'. Regular disease surveys by agronomists, plant pathologists and provincial agricultural staff are necessary to monitor the disease. A better understanding of the benefits of stripe rust resistant varieties and fungicides is possible when the extent of problem is known.

CHAPTER 4. RACES OF *Puccinia striiformis* f. sp. *tritici* IN WESTERN CANADA

4.1. Preface

Previous studies have reported on the epidemiology and virulence phenotypes/races of *Pst* worldwide (Chen, 2005; Sharma-Poudyal *et al*., 2013). The races of *Pst* are characterized in almost every region of wheat cultivation. In Canada, stripe rust of wheat is a relatively new disease compared to leaf and stem rust. The population of *Pst* in Canada was characterized in central Alberta on a local scale (Holtz *et al*., 2013; Kumar *et al*., 2012) and a study was conducted in western Canada many years ago (Su *et al*., 2003). There is no report of the population structure of *Pst* from Saskatchewan or southern Alberta. Regular monitoring of the pathogen population is necessary to support breeding programs and successful management of the disease. The objective is to determine the population structure of *Pst* in Saskatchewan and southern Alberta. The results of the race structure characterization of *Pst* from Saskatchewan and southern Alberta are discussed in this chapter.

4.2. Introduction

Puccinia striiformis Westend. is a basidiomycete, obligate biotrophic pathogen causing stripe rust (yellow rust) of small grain cereals and grasses. *Puccinia striiformis* is further divided into special forms (i.e. formae speciales) based on infective and reproductive capacity on different host genera (Hovmøller *et al*., 2011). Stripe rust of wheat is caused by *Puccinia striiformis* f. sp. *tritici* Eriks. (*Pst*), it occurs everywhere wheat is grown (Hovmøller *et al*., 2011) and may cause significant yield and quality losses (Chen, 2005). Isolates of these special forms are further divided into races or pathotypes based on their reaction on different wheat genotypes referred to as differential wheat lines (Line, 2002).

All individuals in a population with same combination of avirulence genes belong to one race and avirulence genes are detected when individuals are exposed to their corresponding resistance genes in the host differentials (Parlevliet, 1985).

Stripe rust in Canada was first observed on grasses in British Columbia in 1916 (Line, 2002) and on *Hordeum jubatum* in Alberta in 1918 (Fraser and Conners, 1925; Line, 2002). In Saskatchewan, stripe rust was first reported in 1928 (Line, 2002) and disease was common on native grass species in southwestern Saskatchewan, Alberta and British Columbia (Stubbs, 1985). In these years, workers in Canada did not consider this disease to be an economically important issue (Line, 2002). The area of greatest concern was southern Alberta, where *Pst* was presumed to survive and stripe rust was a major production problem (Conner *et al*., 1988; Sanford and Broadfoot, 1932). Stripe rust was a major concern to wheat growers in Alberta but was absent in the eastern prairies until 2000 (McCallum *et al*., 2003, 2004). When stripe rust appeared in the eastern prairies (McCallum and Fetch, 2001), the virulence spectrum was wider (new races were virulent on more differentials) than it was among isolates collected before 2000 (Su *et al*., 2003). Since 2000, the disease has become a regular pest of wheat in Saskatchewan and Manitoba (McCallum *et al*., 2003, 2004). Epidemics of wheat stripe rust were reported in the 1990s (Kumar *et al*., 2012) and an epidemic in a wheat nursery in Alberta in 2005 caused 100% yield loss (McCallum *et al*., 2006) and another epidemic was reported in central Saskatchewan in 2006 (McCallum *et al*., 2007c). Severe stripe rust was recorded throughout southern Alberta and Saskatchewan in 2011 and yield losses up to 35% were recorded (Kutcher *et al*., 2012). Stripe rust was common in Saskatchewan in 2013, although few severely diseased crops were observed (Brar *et al*., 2014).

Puccinia striiformis is reported to overwinter in the Pacific Northwest (PNW) of the United States and wind blown urediniospores initiate epidemics in western Canadian wheat crops (Chen, 2005). There are reports of overwintering of this pathogen in Alberta (Conner *et al*., 1988; Kumar *et al*., 2013); however, the occurrence of *Pst* in Saskatchewan could be the result of *Pst* spores moving along the 'Puccinia Pathway' i.e. the south central USA and not just the PNW of the USA. The races of *Pst* were studied in central Alberta recently, and population structure is somewhat similar to the PNW of the USA (Kumar *et al*., 2012; Holtz *et al*., 2013).

Cultivation of varieties resistant to stripe rust is one of the most efficient disease management methods, as well as an environmentally friendly approach (Kumar *et al*. 2012; Wan and Chen, 2014; Xi *et al*. 2015). However, forces such as mutation or somatic hybridization changes the pathogen population (Hovmøller *et al*. 2011) and selection pressure on favorable mutants or variants give rise to new races. New races in this pathogen develop very rapidly and resistant varieties become susceptible after every few years (Chen, 2005; Kutcher *et al*. 2012). Emergence of highly aggressive races which were, adapted to warmer temperatures (Milus *et al*. 2006, 2009), and virulent on *Yr6*, *Yr7*, *Yr8*, *Yr9* and *Yr10* appeared after the year 2000 making some resistant cultivars susceptible (Chen, 2005; Wan and Chen, 2014). The application of fungicides is required for successful disease management in case of susceptible variety cultivation (Wan and Chen, 2014). Thus, to know about host resistance and successful disease management, the virulence spectrum of the pathogen must be known by monitoring the pathogen population on a regular basis (Su *et al*. 2003; Wan and Chen, 2014).

The present study was designed to study the virulence phenotypes of the population of *Pst* sampled from Saskatchewan and southern Alberta, plus a few isolates from other cropping regions of western Canada. The objectives were: (1) to assess the population structure of *Pst* across western Canada; (2) to investigate virulence diversity, their combinations (races) in western Canada; and (3) to compare races prevalent in different prairie provinces of western Canada.

4.3. Theory

4.3.1. Gene-for-gene theory

The gene-for-gene theory was proposed by Flor (1947) as a result of studies of flax and the flax-rust interaction. This theory states that, for each resistance gene in the host (triggering defense mechanism) there is a corresponding gene for avirulence in the parasite. It means the inheritance of resistance in the host and pathogenicity in the parasite is controlled by pairs of matching genes and these genes in either member are identified when exposed to its counterpart in the other member in the host-pathogen interaction (Flor, 1955). The individuals in a population with the same combination of avirulence genes belong to the same race or pathotype (Parlevliet, 1985).

4.3.2. Discriminant analysis of principal components (DAPC)

Discriminant analyses of principal components (DAPC) is performed in the '*Adegenet*' package of the R software (Jombart, 2013) to identify and describe genetic clusters. In population genetics study, most of the time we are not only interested in studying variation among the individuals but also the diversity among the groups of individuals. Principal component analyses only considers total variation (among and within group variation);

however, DAPC optimizes variation among groups, while minimizing variation within groups. The synthetic variables, i.e. discriminant functions, show differences among groups while minimizing variation within groups (Jombart, 2013).

For DAPC, the groups should be defined prior to analyses, which is achieved by *k*-means, a clustering algorithm that finds a given number (K) of groups or clusters explaining maximum variation among groups. Sequential running of this algorithm and comparing different solutions with Bayesian Information Criterion (BIC) helps to identify the optimal number of clusters or groups. The optimal number of clusters correspond with the lowest BIC, and in practice, the best BIC is indicated by an elbow in the BIC curve. The DAPC is run after principal component analyses of the raw data in the R environment (Jombart, 2013).

4.3.3. Population diversity indices

Total diversity or variation (γ-diversity) in a pathogen population can be expressed as the total of within population diversity (α-diversity) and the diversity between or among populations (β-diversity). In ecological terms, diversity and distance among populations is considered synonymous. This may not be the same because the methods used to calculate distance and diversity among populations may be different. Different parameters can be used to calculate diversity and distance among populations plus diversity within a population (Kosman, 2014; Kosman, 1996). Different diversity indices were used for diversity analyses within populations and distance analyses among populations in the present study. These indices are: Simpson index (Si), normalized Shannon index (Sh), Stoddart index (St), Shannon index (SH), Evenness index (E), Gleason richness index (G),

Kosman diversity within population (KW_m) with regard to simple mismatch dissimilarity, and Kosman distance between regions and clusters (KB) (Kosman, 2014; Schachtel *et al*., 2012; Kosman and Leonard, 2007).

Kosman diversity and Kosman distance are based on dissimilarity between individuals ranging between 0 and 1. Kosman diversity also includes richness (number of races/ number of isolates) and evenness (evenness of race distribution or similarity of race frequencies) parameters of a population in addition to differences among races. Simpson index, Stoddart index, Evenness index and Shannon normalized index are based on frequencies of races. Stoddart index and Simpson index takes richness and evenness of population into consideration, respectively. The Gleason index is sensitive to richness of diversity in the population, whereas the Shannon index reflects both richness and evenness of diversity. Evenness index considers evenness in a population for calculation of an estimator (Kosman and Leonard, 2007). Except for the Shannon, Gleason richness, and Stoddart index, all other indices range between 0 and 1 (Schachtel *et al*., 2012). Diversity in a population is directly proportional to the diversity index (Kosman, 1996). Shannon and Gleason indices treat non-identical races as equally distinct from each other, ignoring virulence similarity. Unlike the Shannon and Gleason indices, the Kosman index takes both race frequency and degrees of similarity in two distinct races into consideration (Kosman and Leonard, 2007, Kosman, 2014).

Formulae used to calculate diversity indices are discussed below:

(i) *Kosman diversity within population (KW_m):*

$$KW_\rho(P) = \frac{1}{n} \cdot Ass^{\rho}_{\max}(P,P).$$

, here P is population of n individuals.

(ii) Gleason richness index (G):

$$G(P) = \frac{s-1}{\ln n}.$$

, here P is population with n individuals and *s* is the total number of types of individuals observed.

(iii) Shannon diversity within population (SH):

$$SH(P) = -\sum_{r=1}^{s} p_r \ln p_r .$$

, here p_r is calculated as n_r/n. n_r is the number of individuals of type T_r.

(iv) Evenness of population (E):

$$E(P) = \frac{SH(P)}{\ln s} = -\frac{1}{\ln s} \cdot \sum_{r=1}^{s} p_r \ln p_r .$$

, ratio of Shannon index to its maximum value *ln s*.

(v) Simpson diversity within population (Si):

$$Si(P) = 1 - \sum_{r=1}^{s} p_r^2 ,$$

(vi) Stoddart diversity within population (St):

$$St(P) = \frac{1}{\sum_{r=1}^{s} p_r^2}.$$

(vii) Normalized Shannon diversity within population (Sh):

$$Sh(P) = \frac{SH(P)}{\ln n} = -\frac{1}{\ln n} \cdot \sum_{r=1}^{s} p_r \ln p_r .$$

4.4. Materials and methods

4.4.1. Field monitoring and sample collection

A total of 61 isolates were collected from diseased wheat plants in commercial fields, and breeding nurseries at various locations in western Canada. More isolates were collected from southern Alberta and Saskatchewan because the population structure of *Pst* in these regions is not well known. Except for one isolate collected from foxtail barley, all other isolates were collected from wheat. Of the 61 isolates, 32 were collected from Saskatchewan, 22 from Alberta, five from Manitoba and two from British Columbia (Appendix I). Nine of these 61 isolates were already characterized in previous studies by Holtz *et a*l. (2013), Kumar *et al*. (2012) and Su *et al*. (2003), although on different differential sets than used in the present study. The majority of the collection was made in years 2011 and 2013. Isolates from the years 1984, 1990, 2005, 2007, 2009, 2010, and 2012 were also included in the study. The information on geographical location, host and year of collection is provided in Appendix-I. Diseased leaf samples were collected, dried in

paper envelopes and stored at 4˚C until further processing. Some isolates were received from colleagues either as pure isolates or a mixture of spores from the field.

4.4.2. Obtaining and storing genetically uniform isolates

'Avocet -*YrA*' wheat, which is susceptible at the seedling stage to all known races of *Pst* in North America, was used to increase urediniospores. Seedlings at the two-leaf stage were inoculated with *Pst* and urediniospores collected from the infected leaves. The inoculated plants were kept in a dew chamber at 10˚C for 48 hours in the dark and then transferred to a growth chamber with diurnal temperature cycle that gradually changed from 12˚C to 17˚C, with a 16 hour photoperiod and 60% relative humidity. Plants inoculated with each sample were placed in a chamber (rectangular cage covered with a plastic sheet) to prevent cross-contamination of the samples. Pustules usually appeared on leaves 14 days post-inoculation (dpi). Single pustule isolates were prepared from the raw samples by transferring a single pustule with a very fine needle to another seedling. This cycle was repeated a number of times to increase the amount of spores required. The urediniospores were collected by tapping infected leaves over aluminum foil. Spores collected were cleaned to remove any dust particles or plant tissue (if any) and cleaned spores were put into Eppendorf tubes. Spores were desiccated using silica at 4˚C for at least three days and then stored at -80˚C until further use.

4.4.3. Screening of isolates

A differential set of 21 wheat lines in the 'Avocet' spring wheat background (Table 4.2), 10 supplemental wheat lines, including some Canadian wheat varieties and 1 triticale variety, were used to characterize the *Pst* isolates. Urediniospores of a single isolate in the amount

of 10 mg were mixed with 950 µl Bayol® 35 (mineral oil) and used to inoculate eight root-trainers constituting two replications of all wheat differentials at the two-leaf stage. This experiment was repeated once. Four pots of 'Avocet –*YrA*' inoculated with one isolate (W010) were also included in each replication as a check throughout all experiments. The check was included to ensure conditions were constant in the growth cabinets for each replication. Statistical analyses on ITs from these check plants did not detect any significant differences among replicates and experiments which, meant that conditions were constant in the growth cabinets. After that, the same protocol was followed as mentioned in Section 4.4.2 up to 14 dpi.

4.4.4. Rating scale

Infection types (ITs) were recorded, in an Excel spreadsheet, 14 dpi using a 0-9 scale (Figure 4.1) modified from that of Line and Qayoum (1992) and McNeal *et al*. (1971). Modification was done in leaf area covered with pustules by gathering information from both scales. The scale is based on leaf surface area covered with pustules or necrotic flecks. The scale was modified in such a way that it is easy to make a base line for classifying isolates as 'Virulent' or 'Avirulent'. The scale has a clear difference in IT of 4 and 5 (Figure 4.1; Table 4.1). Because the modified scale combines the percent of leaf area affected and host response, in case of any discrepancy between two parameters, both were taken into consideration and with the expertise of colleagues to assign the IT.

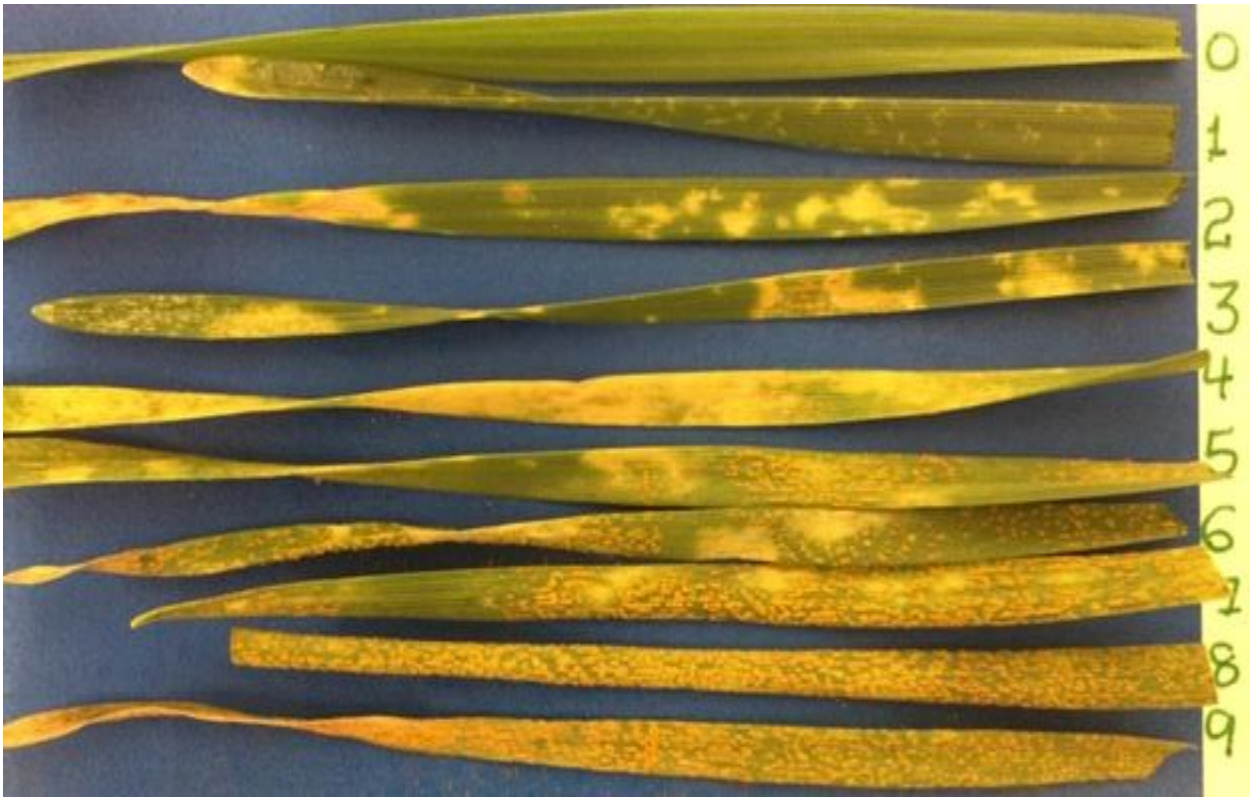


Figure 4.1. Ten category (0-9) rating scale used to classify stripe rust infection types (ITs).

Table 4.1. Stripe rust seedling infection rating scale.

Infection type (IT)	Description	Host response	%Leaf area covered with pustules
0	No visible signs or symptoms	Immune	0
1	Traces of necrotic and/or chlorotic specks; no sporulation	Highly resistant	0
2	Necrotic and/or chlorotic blotches or stripes; no sporulation	Highly resistant to resistant	0
3	Necrotic and/or chlorotic blotches or stripes; trace sporulation	Resistant	<5%
4	Necrotic and/or chlorotic blotches or stripes; light sporulation	Resistant to moderately resistant	5-10%
5	Necrotic and/or chlorotic blotches or stripes; intermediate sporulation	Moderately resistant to moderately susceptible	10-30%
6	Necrotic and/or chlorotic blotches or stripes; moderate sporulation	Moderately susceptible	30-60%
7	Necrotic and/or chlorotic blotches or stripes; abundant sporulation	Susceptible	60-90%
8	Light chlorosis behind sporulating area; abundant sporulation	Highly susceptible	90-100%
9	No chlorosis or necrosis; abundant sporulation	Highly susceptible	100%

4.4.5. Detecting races, their distribution, frequency and diversity

An isolate was considered avirulent between ITs 0-4 (inclusive) and virulent between 5-9 (inclusive). Virulence profiles of *Pst* isolates were converted into 0 (avirulent) and 1 (virulent). Cluster analysis was performed using binary virulence profiles of all isolates. A dendrogram was generated using unweighted pair grouping by mathematical average algorithms (UPGMA) on the basis of pair-wise similarity with simple matching similarity coefficients in sequential, agglomerative, hierarchical and nested (SAHN) clustering method. The analysis was done in numerical taxonomy and multivariate analysis system (NTSYS) software (version 2.2) (Rohlf, 2000). Races were named C-PST-#, where 'C' stands for Canada and 'PST' after the Latin name of the fungus. Races were numbered in order of decreasing number of isolates in each race.

Discriminant analyses of principal components, implemented in the ADEGENET package in the R environment (Jombart *et al*., 2010) were performed to infer the clustering pattern of isolates, based on the virulence phenotypes to assess the grouping of isolates in relation to their geographical location. The number of clusters was selected based on the Bayesian Information Criterion (BIC), as suggested by Jombart *et al*. (2010).

Diversity analyses were performed using the Virulence Analysis Tool (VAT) (Schachtel *et al*., 2012). For diversity analyses, clusters generated by DAPC were considered to calculate diversity within each cluster and isolates were grouped based on the region of collection. Diversity of isolates within regions was examined. Diversity or distance among regions and clusters were also calculated. Diversity analyses for regions

were performed only for Saskatchewan and Alberta because there were relatively few isolates from elsewhere.

4.4.6. Analytical approaches for analyses of virulence data

Race structure studies for wheat rusts are performed using a set of differential host lines. Differential host lines can be established commercial cultivars (differing in genetic background for resistance to the pathogen) or near-isogenic lines (Knott, 1989). The IT recorded for each differential line challenged with each isolate. Infection type of every isolate is converted into binary data and qualitative analysis of the virulence profiles is conducted. This is the most common method of analyzing virulence data for determining races in *Pst*. In addition to the qualitative analysis approach for virulence data, quantitative analysis was conducted in two different ways.

Firstly, IT for each isolate was divided into three classes: IT ranging between 0-3 (resistant/avirulent reaction), 4-5 (intermediate reaction), and 6-9 (susceptible/virulent reaction). A similarity dendrogram was constructed using Gower's general similarity coefficient implemented in the software Multivariate Statistical Package (MVSP version 3.1) (Kovach, 2007).

Secondly, the average ITs for all replications was considered and analyzed without any further division into classes. All 10 categories of ITs ranging from 0-9 were used for quantitative analysis.

4.5. Results

4.5.1. Multivariate analyses of virulence profiles

Cluster analyses detected four major groups of isolates (G1-G4) based on their virulence profiles (Fig. 4.2). Races in each group were independent of their collection location. Group G4 had only one isolate representing one race and was distantly related to groups G1, G2 and G3 (Fig. 4.2). Races in groups G1 and G2 were different from G3 and G4 in terms of virulence spectrum and year of detection. Races in G1 and G2 have a relatively narrow virulence spectrum and all races were detected before or in 2011. Most of the races in Group G3 were detected after 2011. The difference between G3 and either G1 and G2 was attributed to the presence or absence of different virulences or their combinations on differentials (Table 4.2). Virulence on *Yr1* and 'Hybrid46' was detected only in one race in Group G3. Virulence on *YrTye* was detected only in two races in Group G3. The race in G4 was avirulent to *Yr8, YrSu, YrTye,* and 'Lillian' (Table 4.2). Races in groups G1 and G2 were avirulent on *Yr10*, *Yr24* and *Yr26*.

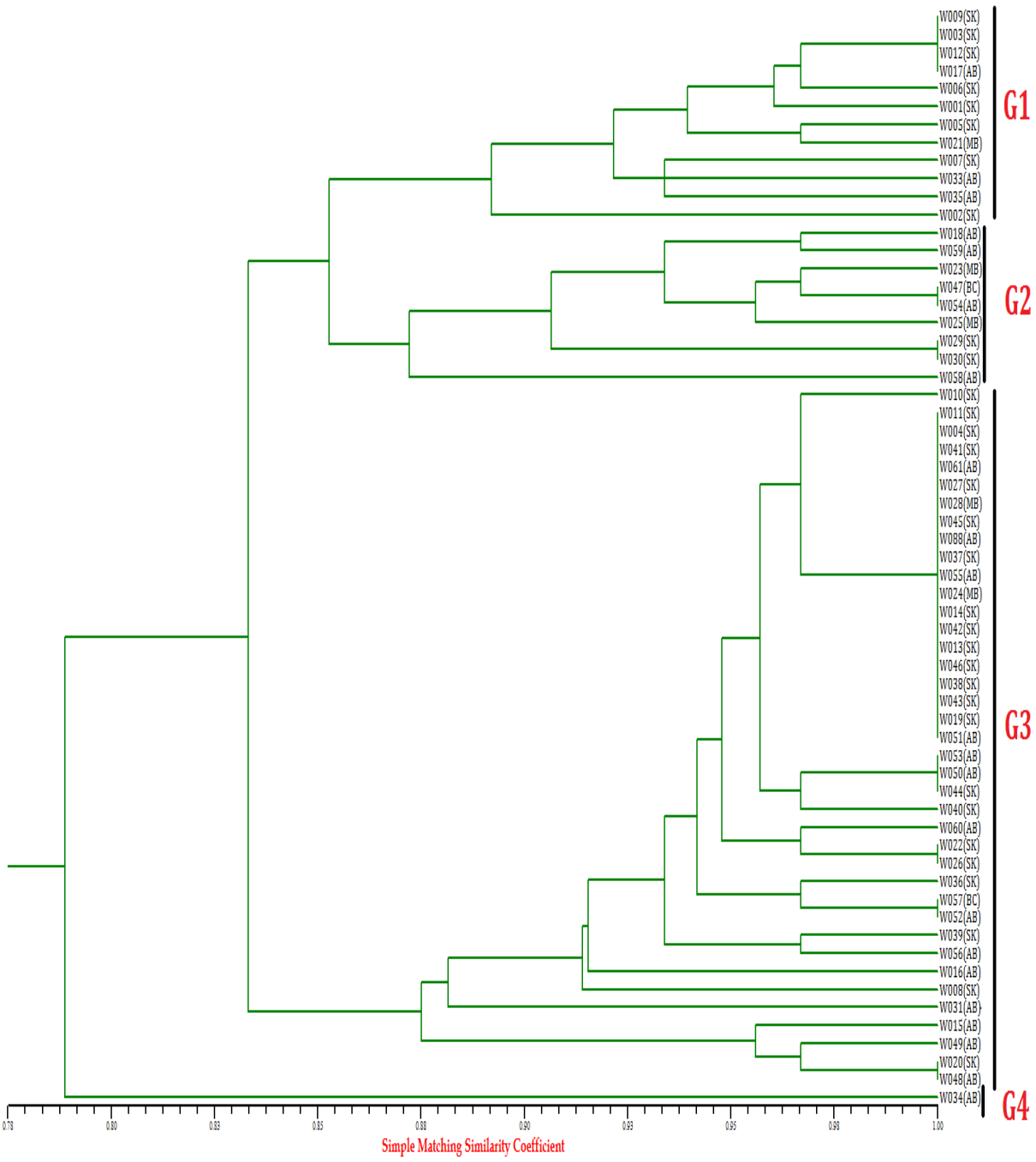


Figure 4.2. Similarity dendrogram based on simple matching coefficient of 61 *Puccinia striiformis* f. sp. *tritici* isolates collected from western Canada based on virulence to wheat differentials and triticale. Vertical lines to the right show the four major groups among 33 races.

Cluster analyses differentiated the 61 isolates into 33 races. Of the 33 races, 25 were represented by only one isolate. In most of the clades, two closely related races differed from each other by a single virulence factor (Appendix I).

The frequency of virulent isolates ranged from 0-100% on 31 wheat differentials (Table 4.2). Differentials 'Avocet -*YrA'*, *Yr6*, *Yr7, Yr9*, *Yr17*, *Yr18*, *Yr28*, and *Yr31* were susceptible to all races. No race was virulent on *Yr5*, *Yr15* or *YrSP.* A virulence frequency of 1.6% was detected for *Yr1* and 'Hybrid46'. Virulence frequency for *YrA*, *Yr2*, *Yr8*, *Yr27*, *Yr29*, *Yr32*, 'Heines 7', 'Nord Deprez' and 'Suwon92*Omar' ranged from 65-98%. The Canadian wheat varieties 'AC Barrie', 'AC Avonlea', and 'CDC Teal' included as supplemental differentials, were susceptible to all races. The virulence frequency on wheat variety 'Lillian' was 63.9% although it is believed to carry at least three APR genes (i.e. *Yr18*, *Yr36,* and *Yr29*). The only triticale variety, 'Brevis' included in the differential set was susceptible to 39.3% of the isolates (Table 4.2). The most common races, C-PST-1 and C-PST-2 belonged to different groups as both are quite different in terms of virulence spectrum.

Table 4.2. Wheat differentials and triticale used to differentiate races of *Puccinia striiformis* f. sp. *tritici*; and virulence frequency of *Pst* on *Yr* genes for all isolates and isolates in individual groups G1-G4 (Figure 4.2).

Differential number	Line/Variety	*Yr* gene present	*Pst* virulence frequency (%)				
			All isolates	G1	G2	G3	G4
1	07YR01	Avocet-*YrA*	100	100	100	100	100
2	07YR02	Avocet+*YrA*	98.4	100	88.9	100	100
3	07YR03	*Yr1*	1.6	0	0	2.6	0
4	07YR04	*Yr2*	96.8	100	77.8	100	100
5	07YR05	*Yr5*	0	0	0	0	0
6	07YR06	*Yr6*	100	100	100	100	100
7	07YR07	*Yr7*	100	100	100	100	100
8	07YR08	*Yr8*	83.6	91.7	22.2	97.4	0
9	07YR09	*Yr9*	100	100	100	100	100
10	07YR10	*Yr10*	18	0	0	25.6	100
11	07YR11	*Yr15*	0	0	0	0	0
12	07YR12	*Yr17*	100	100	100	100	100
13	07YR13	*Yr18*	100	100	100	100	100
14	07YR14	*Yr24*	8.2	0	0	10.3	100
15	07YR15	*Yr26*	9.8	0	0	12.8	100
16	07YR16	*Yr27*	96.8	100	77.8	100	100
17	07YR17	*YrSP*	0	0	0	0	0
18	07YR18	*Yr32*	78.7	8.3	77.8	94.9	100
19	07YR19	*Yr28*	100	100	100	100	100
20	07YR20	*Yr29*	100	100	100	100	100
21	07YR21	*Yr31*	100	100	100	100	100

22	AC Barrie	Unknown	100	100	100	100	100
23	CDC Teal	*Yr18+*	100	100	100	100	100
24	Lillian	*Yr18*, *Yr36+*	63.9	16.7	0	97.4	0
25	Tyee	*YrTye*	3.2	0	0	5.1	0
26	Heines 7	*Yr2, YrVII, Yr25, Yr11*	77.1	83.3	11.1	7.7	100
27	Chinese 166	*Yr1+*	1.6	0	0	2.6	0
28	Nord Deprez	*Yr3a*, *Yr4a*	80.3	25	88.9	94.9	100
29	AC Avonlea	Unknown	100	100	100	100	100
30	Hybrid 46	*Yr3b*, *Yr4b, YrH46*	1.6	0	0	2.6	0
31	Suwon92* Omar	*YrSu*	65.6	8.3	22.2	94.9	0
32	Brevis (triticale)	Unknown	39.3	66.7	0	10.3	0

Among the 33 races detected from the 61 isolates, the most common race, 'C-PST-1' was represented by 19 isolates (31%) (Appendix I), which were avirulent on *Yr1*, *Yr5*, *Yr10*, *Yr15*, *Yr24*, *Yr26*, *YrSP*, *YrTye*, and 'Hybrid46'. The second most common race 'C-PST-2' was represented by four isolates (7%) and was avirulent on *Yr1*, *Yr5*, *Yr10*, *Yr15*, *Yr24*, *Yr26*, *Yr32*, *YrSP*, *YrSu*, *YrTye*, 'Hybrid 46', 'Nord Deprez', and 'Lillian' (Table 4.3). The only isolate collected from foxtail barley belonged to race 'C-PST-8' along with one isolate collected from wheat.

None of the isolates sampled from Saskatchewan, Manitoba and British Columbia were virulent on *Yr1*. Isolates virulent on *Yr10*, *Yr24*, *Yr26* and *YrTye* were not detected in Saskatchewan until 2013 (Table 4.3). Virulence for *Yr10* was common in isolates sampled from southern Alberta. A race from SK with virulence on 'Hybrid 46' was detected in 2011

but was not recovered in 2013. Race 'C-PST-21' (detected in 2007) was represented by only one isolate and was virulent on the fewest differentials (Table 4.3).

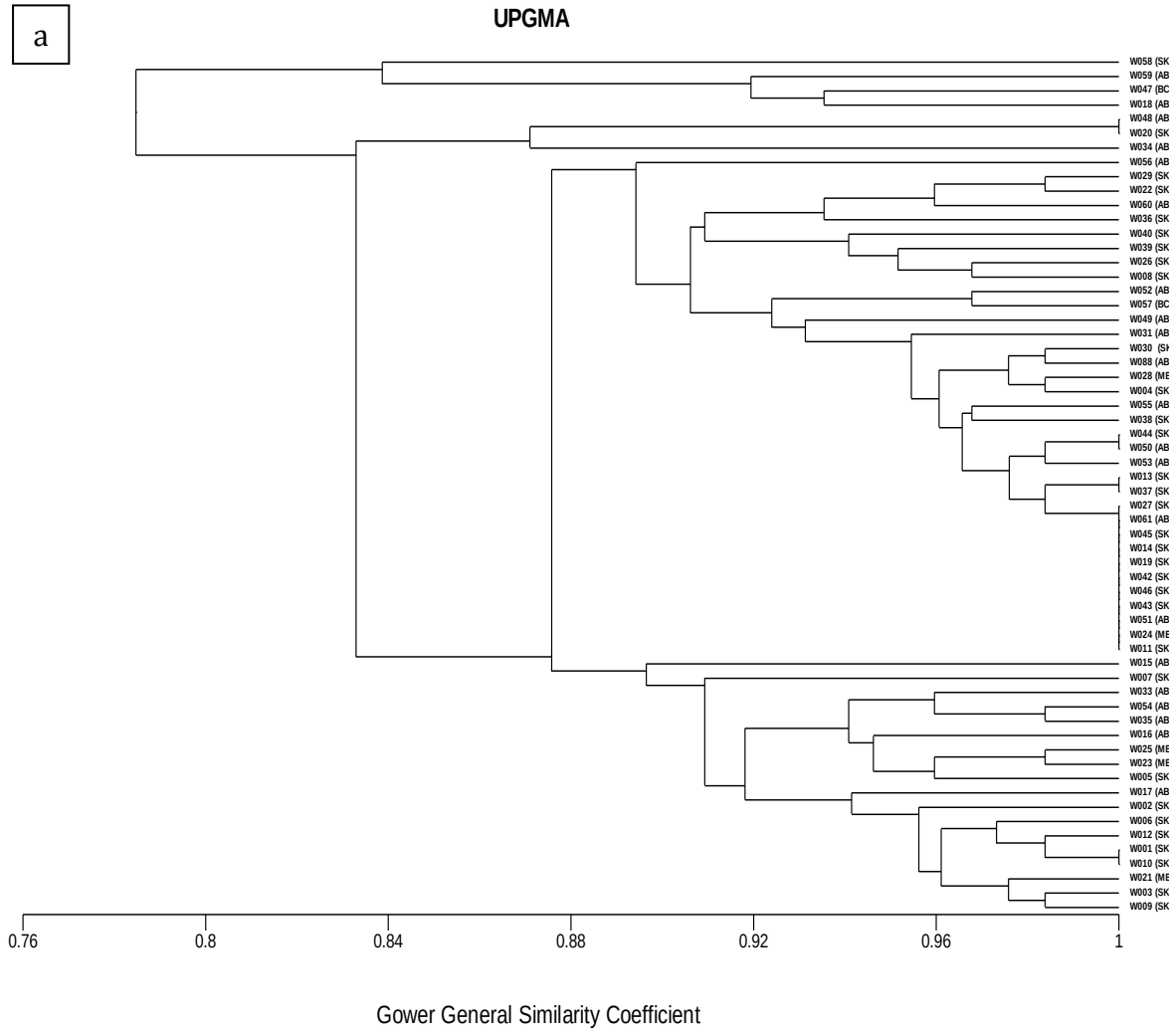
Nine of the 61 isolates included in the present study, were already characterized by Su *et al*. (2003), Kumar *et al*. (2012) and Holtz *et al*. (2013) on different differentials. The results were compared on differentials in common among all studies. The reactions of two *Pst* isolates, W047 and W088 included from the study by Su *et al*. were similar for common differentials, although isolate W088, which was reported to be avirulent on *Yr32*(=*CV*), was virulent on this differential in the study. One isolate (W057) was included from the study by Kumar *et al*. (2012), which had similar reaction on all differentials except *Yr26* and *Yr28*. This isolate was virulent on *Yr26* and avirulent on *Yr28* in the Kumar *et al*. (2012) study, which was opposite to what was observed in the present study. Six isolates: W031, W033, W034, W035, W055 and W056 were included from the study by Holtz *et al*. (2013). Isolates W034 and W035 gave similar results on all common differentials in both studies, but the other isolates differed in their reaction on *Yr28*. These were reported as avirulent whereas the present study found these six isolates virulent on *Yr28*. The variation in reaction, among studies, for these isolates can be attributed to differences in disease assessments, differences in experimental conditions and in rating scales.

4.5.2. Quantitative analyses of virulence profiles

Sixty-one *Pst* isolates were differentiated into 46 races (Figure 4.3a). The most common race was represented by 11 isolates (18%) of the total. Four races were represented by two isolates in each and the rest of the races were unique. The step-wise gain in virulence for

two closely related races could not be explained in this case unlike qualitative analysis of virulence profiles.

A similarity dendrogram from on ten categories based on Gower's general similarity coefficient for 61 isolates is showed in Figure 4.3b Using this analysis every single isolate represented a unique *Pst* race.



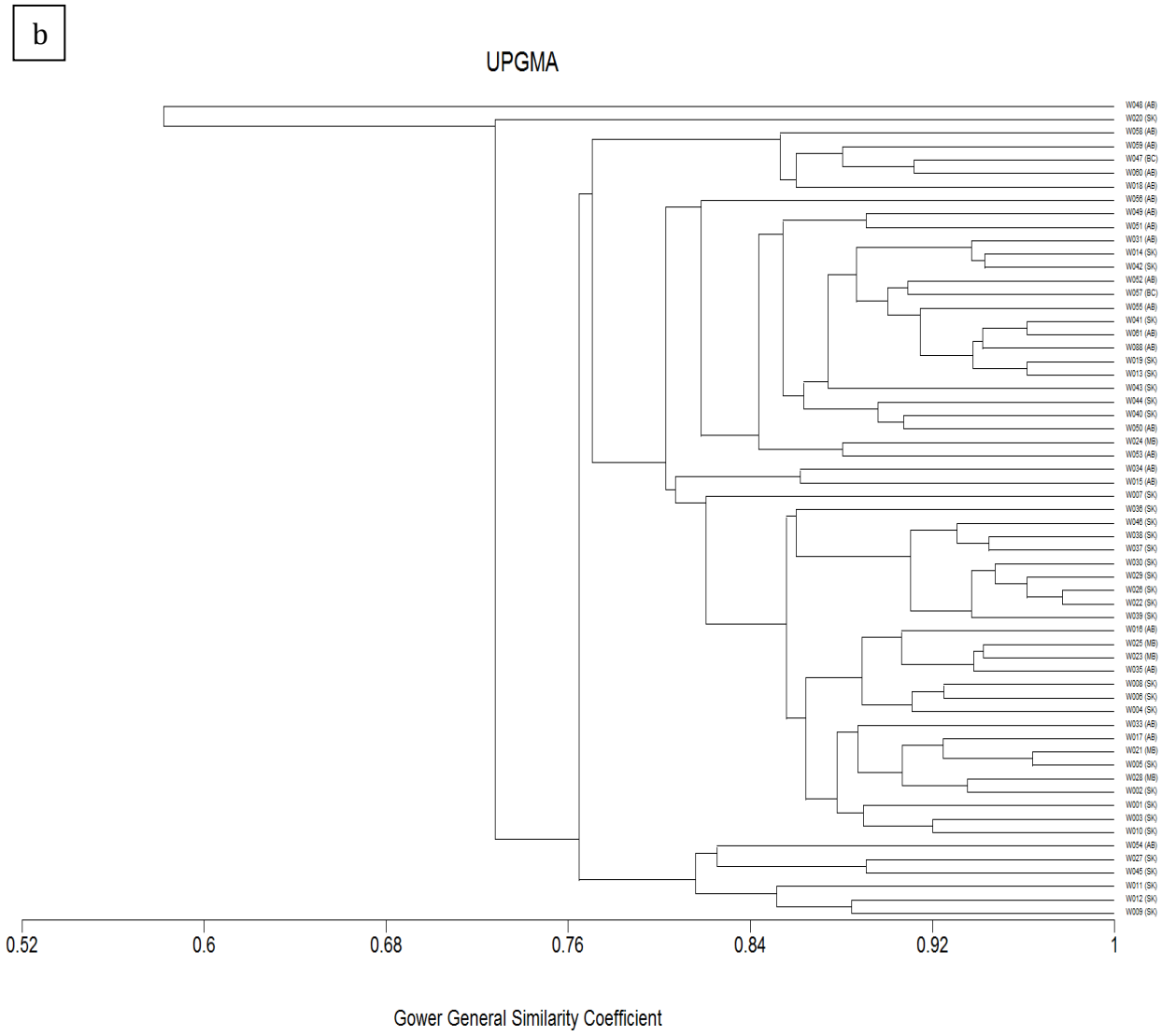


Figure 4.3. Similarity dendrogram based on Gower's general similarity coefficient (a) for three categories for IT (b) for 10 categories, of 61 *Puccinia striiformis* f. sp. *tritici* isolates collected from western Canada based on virulence to wheat differentials and triticale.

Quantitative analyses of virulence profiles explains more variation in the pathogen population; however, it is difficult for researchers to draw conclusions using this kind of data. The purpose of virulence characterization is to explain the effectiveness of resistant genes in wheat germplasm. The pathogen race with frequent occurrence should be known

because if the most common race is virulent on an elite wheat variety (favored by growers and with good marketing potential), wheat breeders can introgress a source of resistance against that race. Some races with wider virulence spectrum but very low frequency are less of a concern than most common races. This information can be better explained with qualitative analysis of virulence profiles as discussed in Chapter 4. Also, quantitative analyses could differ from person to person as disease rating is subjective in nature, but this variation is expected to be less of a concern when classifying the reaction into race, two categories: Resistance or Susceptible.

Note: Only qualitative analysis of virulence profiles is considered to draw further conclusions in this Chapter.

4.5.3. Discriminant analyses of principal components

The non-parametric discriminant analyses of principal components (DAPC) of virulence profiles of *Pst* isolates identified at least three groups or clusters (K=3) (Figures 4.4 and 4.5), supported by the Bayesian Information Criterion (BIC) curve (Figure 4.6). The clusters are based on races rather than their collection locations.

The three clusters identified by DAPC are similar to the groups in Fig. 4.2 except for Group G4, which was merged in Cluster 2. Clusters 1 and 2 represent races with relatively narrow virulence spectrum. These were detected in or before 2011. Figure 4.5 represents tabular form of the clusters with different races. Heat colors in Fig. 4.5 represent the membership probability of each race (red=1, white=0). Blue crosses in each cell represent the prior cluster provided to DAPC. In this case, from all races, DAPC classification is consistent with the original clusters.

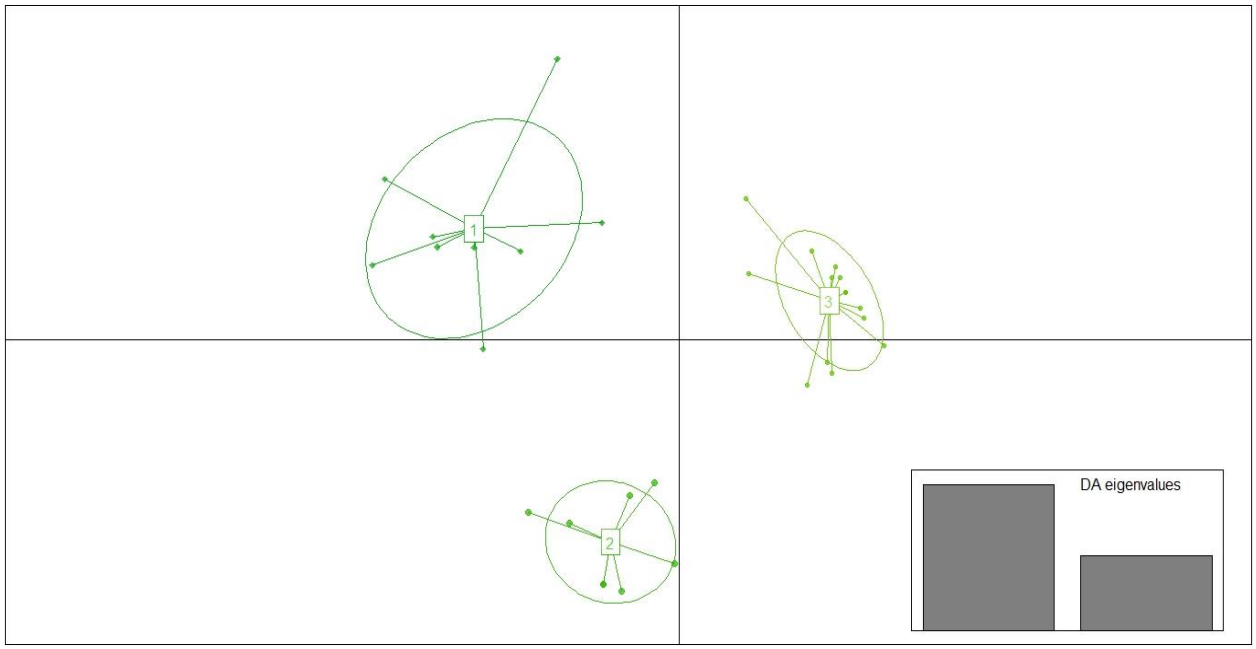


Figure 4.4. Discriminant analyses of principal components (DAPC) for 33 *Puccinia striiformis* f. sp. *tritici* races detected in western Canada, suggesting at least three sub-populations. The bar-plot in the bottom right-hand corner represents the DA eigenvalues for two main principal components/discriminant functions (41% and 20% of the total variation is explained by the two discriminant functions).

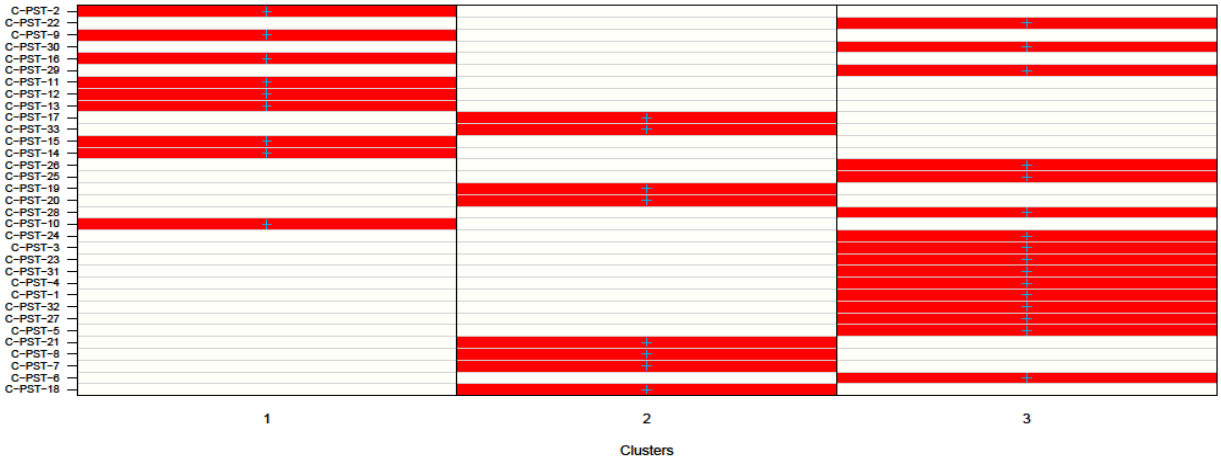


Figure 4.5. Tabular form of the three clusters of races of *Puccinia striiformis* f. sp. *tritici* identified by DAPC. Races detected in western Canada and heat colors in each cell with membership probability (represented by blue crosses) assigned each race to a cluster.

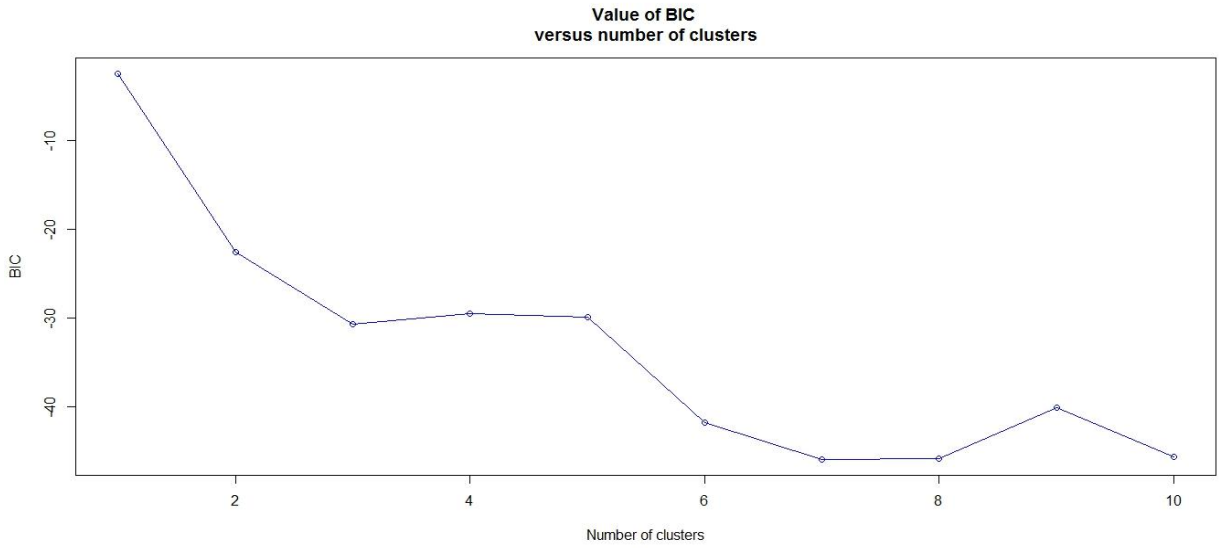


Figure 4.6. Bayesian Information Criterion (BIC) curve suggesting the minimum number of clusters (K) required to explain the variation between race clusters.

4.5.4. Diversity analyses

The diversity of the 61 *Pst* isolates examined can be divided into sub-populations based on differences in virulence (Tables 4.4 and 4.5). Diversity of the *Pst* population in AB is different than the SK population and regardless of the index used, diversity of the AB population is greater than that of the SK population. There is more diversity in Cluster-2 (G2+G4) than Cluster-1 (G1) and Cluster-3 (G3) (Table 4.4), except for the Gleason richness and Shannon indices, which indicated that Cluster-3 is the most diverse. The diversity between each cluster and group was not great (0.134-0.163) (Table 4.5). Diversity between *Pst* populations in SK and AB was small, as indicated by the Kosman distance (KB) index (Table 4.5).

Table 4.3. Virulence spectra and frequency of *Puccinia striiformis* f. sp. *tritici* races sampled from 1984-2013 in western Canada.

Pst race	Year of 1st detection	Region of 1st detection	Avocet -*YrA*	*Yr A*	*Yr 1*	*Yr 2*	*Yr 5*	*Yr 6*	*Yr 7*	*Yr 8*	*Yr 9*	*Yr 10*	*Yr 15*	*Yr 17*	*Yr 18*	*Yr 24*	*Yr 26*	*Yr 27*	*Yr SP*	*Yr 32*	*Yr 28*	*Yr 29*	*Yr 31*	*Yr Su*	*Yr 3b,4b*	*Yr 3a,4a*	*Yr 18+36+*	*Yr 2+HVII+25*	*Yr Tye*	AC Barrie	CDC Teal	AC Avonlea	Brevis (Trit.)	Race Freq.
C-PST-1	1984	AB	+[a]	+	-[b]	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	-	31.1
C-PST-2	2010	AB	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	-	+	+	+	-	-	-	-	+	-	+	+	+	+	6.6
C-PST-3	2012	AB	+	+	-	+	-	+	+	+	+	+	-	+	+	-	-	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	4.9
C-PST-4	2013	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	3.3
C-PST-5	2009	BC	+	+	-	+	-	+	+	+	+	+	-	+	+	-	-	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	-	3.3
C-PST-6	2013	AB, SK	+	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	3.3
C-PST-7	1984	BC	+	+	-	+	-	+	+	-	+	-	-	+	+	-	-	+	-	+	+	+	+	-	-	+	-	-	-	+	+	+	-	3.3
C-PST-8	2013	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	-	+	-	-	-	+	+	+	-	3.3
C-PST-9	2011	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	-	+	+	+	-	-	-	+	+	-	+	+	+	+	1.6
C-PST-10	2011	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	-	+	+	+	+	-	-	-	+	-	+	+	+	+	1.6
C-PST-11	2011	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	-	+	+	+	-	-	+	-	+	-	+	+	+	+	1.6
C-PST-12	2005	MB	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	-	+	+	+	-	-	+	-	+	-	+	+	+	-	1.6
C-PST-13	2011	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	-	+	+	+	-	-	-	-	-	-	+	+	+	-	1.6
C-PST-14	2011	AB	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	-	+	+	+	-	1.6
C-PST-15	2011	AB	+	+	-	+	-	+	+	-	+	-	-	+	+	-	-	+	-	-	+	+	+	-	-	-	-	+	-	+	+	+	-	1.6
C-PST-16	2011	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	-	+	+	+	-	-	+	+	-	-	+	+	+	+	1.6
C-PST-17	2011	AB	+	+	-	+	-	+	+	-	+	-	-	+	+	-	-	+	-	-	+	+	+	-	-	+	-	-	-	+	+	+	-	1.6
C-PST-18	2011	AB	+	+	-	-	-	+	+	-	+	-	-	+	+	-	-	-	-	-	+	+	+	-	-	+	-	-	-	+	+	+	-	1.6
C-PST-19	2005	MB	+	+	-	+	-	+	+	-	+	-	-	+	+	-	-	+	-	+	+	+	+	-	-	+	-	+	-	+	+	+	-	1.6
C-PST-20	2005	MB	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	-	-	-	-	-	-	+	+	+	-	1.6
C-PST-21	2007	AB	+	-	-	+	-	+	+	-	+	-	-	+	+	-	-	-	-	+	+	+	+	-	-	+	-	-	-	+	+	+	-	1.6
C-PST-22	2011	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	-	+	+	+	+	-	+	+	+	-	+	+	+	-	1.6
C-PST-23	2013	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	-	-	+	+	-	+	+	+	+	1.6
C-PST-24	2013	AB	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	-	1.6
C-PST-25	2013	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	1.6
C-PST-26	2013	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	1.6
C-PST-27	2011	AB	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	1.6
C-PST-28	2011	AB	+	+	-	+	-	+	+	-	+	-	-	+	+	-	-	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	1.6
C-PST-29	2011	SK	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	1.6
C-PST-30	2010	AB	+	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	1.6
C-PST-31	2013	AB	+	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	-	+	+	+	+	1.6
C-PST-32	2011	AB	+	+	-	+	-	+	+	+	+	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	+	+	-	+	+	+	+	1.6
C-PST-33	2011	AB	+	+	-	+	-	+	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	-	-	+	-	+	-	+	+	+	-	1.6

[a]Virulence reaction; [b]Avirulence reaction

Table 4.4. Diversity indices within the population of *Puccinia striiformis* f. sp. *tritici*.

Diversity Index	Alberta	Saskatchewan	Cluster-1[a] (G1)	Cluster-2 (G2+G4)	Cluster-3 (G3)
Simpson index (Si)	0.926	0.807	0.833	0.860	0.742
Normalized Shannon index (Sh)	0.898	0.645	0.814	0.880	0.556
Stoddart index (St)	13.444	5.172	6.000	7.143	3.870
Shannon index (SH)	2.776	2.234	2.023	2.025	2.038
Evenness index (E)	0.960	0.806	0.921	0.974	0.735
Gleason richness index (G)	5.500	4.328	3.219	3.040	4.094
Kosman index (KW_m)	0.207	0.096	0.073	0.106	0.066

[a]Clusters and groups in reference to DAPC and NTSYSpc results (Figures 4.2, 4.3, and 4.4)

Table 4.5. Kosman distance (KB) among *Puccinia striiformis* f. sp. *tritici* sub-populations (clusters) and regions.

Regions/clusters	Kosman distance (KB) index
Alberta-Saskatchewan	0.065
Cluster1[a] -Cluster2 (G1 and G2+G4)	0.140
Cluster2-Cluster3 (G2+G4 and G3)	0.163
Cluster1-Cluster3 (G1 and G3)	0.134

[a]Clusters and groups in reference to DAPC and NTSYSpc results (Figures 4.2, 4.3, and 4.4)

4.6. Discussion

The majority of races in the present study differ from other races by a single virulence or avirulence factor, where virulence is added or lost in a step-wise fashion. This supports the theory of stepwise addition or loss of virulence contrary to somatic recombination. New races of *Pst* are believed to evolve from stepwise mutation, rather than rare events like somatic recombination (Hovmøller *et al*., 2002; Wellings and McIntosh, 1990). If somatic recombination were common, the new races would be expected to have a combination of

virulences (Steele *et al*., 2001). There are a few races showing weak evidence of virulence recombinations from co-existing races (data not shown).

The relatively narrow virulence spectrum and the high frequency of the most common race detected in western Canada (C-PST-1), in comparison with other races with wider virulence spectrum, but very low frequency, could be the result of a trade-off between virulence and fitness/aggressiveness in the pathogen meta-populations (Thrall and Burdon, 2003).

The genes *Yr15* and *YrSP* were effective against all races of *Pst,* and *Yr1* was defeated by only one race (Table 4.3). A race virulent on *Yr1* was first reported in Alberta in 1989 (Su *et al*., 2003). Races virulent on *Yr15* and *YrSP* were reported for the first time in 1990 in Alberta and again in 1991 and 1995, but again the frequency was very low (Su *et al*., 2003). The low frequency and instability of races virulent on these genes indicate that these races were not common in the *Pst* population prevalent in western Canada. This might be associated with the lack of selective advantage for those virulent races. If these genes are not carried by commercial wheat varieties in Canada, there would be no selection pressure on *Pst* races for virulence on these genes.

Avirulence on *YrA*, *Yr2*, *Yr8*, and *Yr27* by two races (C-PST-18 and C-PST-21, Table 4.3) was observed, although these genes are defeated by races prevalent in western Canada. These races might be old (races detected before 2000) that are present in very low frequency, or they could be the result of back-mutations to virulence on *YrA* and *Yr2*. Back-mutations were observed previously in *Pst* for genes *Yr9* and *Yr32* (Hovmøller *et al*., 2007) and for *YrA* (Wellings and McIntosh, 1990). All races detected after 2000 in western Canada

are virulent on genes *Yr6*, *Yr7*, *Yr8* and *Yr9* except for a very few races, which are avirulent to *Yr8*. Virulence to *Yr6* and *Yr7* is common and could be a result of races arrived from Asia and Africa where these genes were deployed in wheat varieties. Gene *Yr6* is present in a wide range of bread and durum wheat varieties in Asia and Europe (Chilosi and Johnson, 1990), which explains the high virulence frequency of all races detected worldwide on *Yr6*. Virulence on *Yr7* can be explained through its use in Thatcher (*Yr7*) and old landraces (Sharma-Poudyal *et al*., 2013). Virulence on *Yr8* and *Yr9* in North America is result of an invasive population that arrived in 2000 (Chen *et al*., 2010). Virulence on *Yr8* and *Yr9* was reported previous to 2000 at only low frequencies (Chen *et al*., 2010; Su *et al*., 2003). The virulence on *Yr8* worldwide can be explained by extreme selection pressure on the pathogen population, as this gene was derived from *Aegilops comosa* and is present in many grass species (Stubbs, 1985). Similarly, *Yr9* originated from rye and was used extensively in breeding programs worldwide, which resulted in the emergence of races virulent on *Yr9* (Sharma-Poudyal *et al*., 2013). The spread of *Pst* races virulent on *Yr8* and *Yr9* to Canada is the result of its existence in the USA.

Genes *Yr24* and *Yr26* are reported to be the same gene (Li *et al*., 2006). All races, except for C-PST-32 (avirulent on *Yr24* and virulent on *Yr26*), in the present study resulted in a slow rusting reaction on *Yr24* and *Yr26.* These genes are typical slow-rusting genes (Colin Wellings, personal communication) and all races except one gave the same reaction. The reaction resulting from race C-PST-32 suggests that these genes may not be the same.

The virulence on *Yr27* was detected in all races, except two from AB, and the same trend was observed for races prevalent in Alberta (Holtz *et al*., 2013; Kumar *et al*., 2012).

The *Yr27* gene was deployed in a wide range of wheat varieties in western Asia and Africa, but the severe epidemic of 2010 (Borlaug Global Rust Initiative, 2010) resulted in resistance breakdown. This gene is linked to leaf rust resistance genes *Lr13* and *Lr23* and also to stem rust resistance gene *Sr10*, which were used extensively in breeding programs (McDonald *et al*., 2004). This could have resulted in the loss of *Yr27* to virulent races due to extreme selection pressure for virulence in *Pst*.

Triticale variety 'Brevis' was included in the differential set because triticale is reported to have resistance to most of the races of *Pst* (Wellings *et al*., 2012). Contrary to expected results, the virulence frequency on 'Brevis' was high (39%). 'Brevis' along with other triticale varieties was reported to be highly resistant to stripe rust in field nurseries at plant maturity in western Canadian in 2011 (Randhawa *et al*., 2012), 2013 and 2014 in stripe rust nurseries in Saskatchewan (Randy Kutcher, personal communication). Because rust inoculations for race characterization were performed at the seedling stage, many races were virulent on triticale, suggesting that there might be some unknown adult plant resistance gene(s) in triticale germplasm in Canada.

The hard red spring wheat variety 'Lillian' was included as a supplemental line in the differential set and known to carry APR genes *Yr18*, *Yr36* and *Yr29* (DePauw *et al*., 2005; Ron DePauw, breeder of the variety, personal communication), which are not expressed at the seedling stage. However, some races were observed to be avirulent on 'Lillian' at the seedling stage, which may indicate that 'Lillian' has an unknown seedling resistance gene(s).

The majority of isolates were avirulent on *Yr10*. Seven of 10 isolates virulent on *Yr10* were recovered from AB and one from BC, but only two from Saskatchewan. This gene is present in Canadian winter wheat variety 'AC Radiant', which became susceptible to stripe rust in 2010 and 2011 in southern AB (Kutcher *et al*., 2012). It suggests races virulent on *Yr10* are not common in the *Pst* population prevalent in SK. The virulence frequency on *Yr10* in Alberta was higher in 2013 (Holtz *et al*., 2013) than reported in 2012 (Kumar *et al*., 2012) and previous years (Su *et al*., 2003). Races virulent on *Yr10* are more common in southern BC (Su *et al*., 2003) and AB (Holtz *et al*., 2013) than in SK and MB. These races might arrive in the air-borne inoculum from the PNW in the USA, where races virulent on *Yr10* occur almost every year but at low frequency (Chen *et al*., 2010).

The pathogen, *Pst,* has potential to overwinter in Alberta, Canada (Conner *et al*., 1988; Kumar *et al*., 2013) and stripe rust was reported early in the season in Alberta in 2014 (D. Gaudet, K. Kumar, personal communication), which was unusual. The growing season in western Canada is short, so planting and harvesting of spring and winter wheat can overlap resulting in a 'green bridge' for *Pst* urediniospores produced from overwintering on winter wheat (Kumar *et al*., 2013). Most of winter wheat varieties are susceptible to stripe rust (Anonymous, 2010a). Most Canadian wheat varieties are deployed with either *Yr18* or *Yr36,* which are APR genes. The *Yr18* gene (linked to *Lr34*) is widespread in Canadian varieties because it conditions leaf rust resistance; however, *Yr18* is not deployed in Canadian western soft white spring (CWSWS) or Canadian western amber durum (CWAD) varieties (Randhawa *et al*., 2012). Most Canadian wheat varieties lack all-stage resistance genes and virulence frequency of *Pst* races on common wheat varieties in Canada is very high (up to 100%) (Table 4.2; Holtz *et al*., 2013; Randhawa *et al*., 2012).

Virulence frequency of *Pst* on *Yr1*, *YrSP*, and *YrTye* in AB and BC is very similar to the virulence frequency prevalent in the PNW, USA (Holtz *et al*., 2013; Kumar *et al*., 2012; Su *et al*., 2003; Wan and Chen, 2014; Wan and Chen, personal communication), whereas the virulence frequency of SK races on these genes is either zero or very low. In addition, virulence on *Yr10*, *Yr26*, *Yr28,* and *Yr32* is different for SK races, as compared with races from AB and BC. Virulence was detected more commonly in PNW races for *Yr1*, *Yr10*, *Yr32*, *YrSP, YrTye* than from states east of the Rocky Mountains (Wan and Chen, 2014). It suggests that the *Pst* population in SK is a result of inoculum coming from the PNW and the 'Puccinia pathway', which can also be explained on the basis of the prevailing winds from west to east and south to north during the summer. Also, races in Great Plains of the USA are more similar (as compared to races from the PNW) to races in SK (Wan and Chen, personal communication). This is also true for other closely related rusts (Leaf and Stem rust) of wheat (Brent McCallum and Tom Fetch, personal communication).

The diversity distance of *Pst* populations prevalent in AB and SK is low (Kosman distance index 0.065) (Table 4.5). This can be attributed to the lack of any geographical barrier in the western Canadian prairies, resulting in a uniform population of *Pst*. The difference in diversity among clusters did not yield any useful information. The different diversity indices suggested that within the *Pst* population, diversity is greater among isolates collected in AB than in SK. This may be attributed to the close proximity of southern Alberta with stripe rust epidemiological Regions 1 and 2 (PNW) in the US, where stripe rust is a major problem and the *Pst* population is very diverse. In addition, there is a greater genetic diversity of *Pst* resistance genes in wheat varieties grown in the PNW resulting in a high frequency of evolution in the *Pst* population in the PNW (Chen *et al*.,

2010). The greater diversity of *Pst* in AB could also be attributed to the existence of races with unique virulence combinations on *Yr1*, *Yr10*, *YrSP* and *YrTye* (Table 4.3 and Holtz *et al*., 2013).

4.7. Conclusions

The present study indicated that the population structure of *Pst* in SK is different than in AB, which may be because the population structure in SK is a result of inoculum arriving via the 'Puccinia Pathway' in addition to inoculum from the PNW and southern Alberta. Although diversity within the *Pst* population in AB is high compared to SK, it is linked with the low diversity of *Pst* in the south-central US compared to the PNW. The high diversity of the *Pst* population in western Canada may result from air-borne inoculum from the USA. The pathogen population evolves rapidly by gaining virulence towards specific resistance genes. The overwintering potential of *Pst* in western Canada, the susceptibility of Canadian wheat varieties to stripe rust at the seedling stage and the 'green bridge' between winter and spring wheat provides perfect conditions for stripe rust epidemics in western Canada.

CHAPTER 5: GENETIC DIVERSITY IN THE *Puccinia striiformis* f. sp. *tritici* POPULATION IN WESTERN CANADA

5.1. Preface

The existence of many physiological races of *Puccinia striiformis* f. sp. *tritici* indicates variation in terms of virulence in western Canada. Different virulence phenotypes will also differ in their genetic make-up or alternatively, genetic variation among individuals may give rise to new physiological races. Traditional approaches use phenotypic markers to characterize the population structure of *Pst*, but recently the development of different kinds of molecular markers provide more tools to understand the genetic make-up of *Pst* populations. This chapter describes the use of genome wide SNPs to understand the population structure of *Pst* in western Canada.

5.2. Introduction

Stripe rust of wheat, caused by *Puccinia striiformis* West. f. sp. *tritici* Erikks. (*Pst*), is one of the most devastating diseases of wheat (Chen, 2005; Hovmøller, 2011; Line, 2002). *Puccinia striiformis* f. sp. *tritici* is an obligate, biotrophic and heteroecious rust fungus in the order Uredinales, phylum Basidiomycota (Stubbs, 1985). The complete life cycle of the fungus was only recently determined with the discovery of alternate hosts (Jin *et al*., 2010; Wang and Chen, 2013). The fungus is divided into special forms (formae speciales) based on infective and reproductive capacity on different host genera (Erikkson, 1894 cited in Hovmøller *et al*., 2011; Wellings *et al*., 2000). These special forms are: *P. striiformis* f. sp. *tritici* on wheat, *P. striiformis* f. sp. *hordei* on barley, *P. striiformis* f. sp. *secalis* on rye, *P. striiformis* f. sp. *elymi* on *Elymus* spp. and *P. striiformis* f. sp. *agropyron* on *Agropyron* spp.

(Hovmøller *et al*., 2011), *P. striiformis* f. sp. *pseudo-hordei* on barley grass (Wellings *et al*., 2000). Among the formae speciales, the *tritici* form that infects wheat is the most important and causes huge losses in quality and quantity of grain (Chen, 2005).

Mutation, somatic recombination, parasexual cycle, selection pressure and the sexual cycle may all be more or less responsible for the genetic variation observed in the *Pst* population (Hovmøller *et al*., 2011). Single point mutations are the most common cause of change from virulence to avirulence and development of new races (Steele *et al.*, 2001). Somatic recombinations are detected in natural *Pst* populations but are uncommon (Hovmøller *et al*., 2011).

Stripe rust is widespread on the Canadian prairies since 2000 and has a relatively wider virulence spectrum than populations prevalent before 2000 ((Kutcher *et al*., 2012; Su *et al*., 2003). In the USA and Canada, most studies on diversity of *Pst* are based on virulence phenotypes (Line, 2002; Stubbs, 1988; Markell and Milus, 2008). Virulence is governed by a few loci in the genome that are under intense selection pressure because of 'race-specific' genes deployed in wheat varieties (Markell and Milus, 2008) and thus their frequency in the field populations is largely governed by man (Michelmore and Hulbert, 1987). Chen *et al*. (1993) were among the first to use molecular markers to study *Pst* populations. RAPD markers clearly distinguished the *Pst* population based on its virulence on *Yr1*. The DNA polymorphism of *Pst* genotypes is usually independent of virulence and sampling locations of the genotypes (Chen *et al*., 1993; Shan *et al*., 1995; Wellings, 2007). Amplified Fragment Length Polmorphism (AFLP) markers were used to determine aerial dispersal of *Pst* in Europe (Justesen *et al*., 2002) and the genetic evolution of the pathogen

was demonstrated in China (Zheng *et al*., 2001). Molecular markers (SSR and AFLP) were used by Markell *et al*. (2004) (cited in Chen, 2005) to distinguish old *Pst* populations (pre-2000) from populations examined after 2000. In Canada, studies on *Pst* population diversity were based mainly on virulence markers (Su *et al*., 2003; Kumar *et al*., 2012; Holtz *et al*., 2013). Only one study in Canada, by Holtz *et al*. (2013) used SSR markers to look at molecular genotypes prevalent in central Alberta.

Polymorphic markers such as RAPDs, AFLPs and SSRs were widely used in *Pst* to study population structure, but the use of recently developed SNP variants/markers is limited in phytopathogenic fungi. The high heritability and abundance of SNPs in both coding and non-coding regions of the genome make it well suited for association and diversity studies (Morin *et al*., 2004). The present study was aimed at determining (i) the population structure of *Pst* prevalent in western Canada using genome-wide SNPs, and (ii) to differentiate the *Pst* populations in the western and eastern prairies of western Canada because it was hypothesized that the population in the west is different from the population in the east in terms of virulence variation and adaptation.

5.3. Theory

5.3.1. Different parameters for testing recombination (implemented in the RDP4 program)

The recombination detection program (Martin *et al*., 2010) uses various methods to detect and characterize recombination events evident within a sequence alignment. Various methods, implemented in RDP4, that were used to detect recombination in the *Pst* population in the present study are described below.

5.3.1.1. The RDP method

The RDP method examines triplets of sequences to detect unique recombination events (Martin and Rybicki, 2000). The same nucleotide sites in each set of triplets are excluded and a window is moved along the informative sites. Potential recombinant regions are identified where parent-recombinant percentage identity is higher than parent-parent percentage identity. After all possible combinations of triplets are examined, the number of unique recombination events are listed. The probability of chances of nucleotide identities is approximated using the binomial distribution. The p-value is calculated by multiplying that probability with the number of windows examined. A multiple-comparison correction (or Bonferroni correction) p-value is calculated from this p-value by multiplying it with the total number of triplets examined within the alignment.

5.3.1.2. GENECONV method

The GENECONV method ignores monomorphic sites such as RDP (Padidam *et al*., 1999; Sawyer, 1989). The method looks for regions within alignments sufficiently similar to suspect recombination events. From triplets of sequences, a pair of sequences is sampled and regions in those sequences are scored. Regions to be scored are found that are either identical and unusually long or unusually similar for that pair of sequences. Similarity is scored based on matches and mismatches. There is a penalty for mismatches, which depends on the density of polymorphic sites between two sequences. These scores are converted into Karlin-Altshul-like p-values and potential recombinants are identified. The problem in using the GENECONV method lies in misinterpretation of conserved sites,

between the sequences, as recombinants. The breakpoint detection accuracy is not reliable in this method compared to other methods.

5.3.1.3. Bootscan/Recscan method

In Bootscan method (Martin *et al*., 2005), a portion of an alignment falling within a specified window is selected. Then bootstrap replicates are made, and midpoint rooted neighbor-joining (NJ) trees are constructed by plotting the relative bootstrap support for nearest neighbor groupings of the potentially recombinant sequence with each of the reference sequences at each window position. Non-recombinant sequences should group (>70% bootstrap) with a single reference sequence whereas recombinant sequences should group alternatively (>70% bootstrap) with two or more different reference sequences. High degrees of bootstrap between two different sequence pairs are indicative of potential recombination events. Either binomial or Chi-squared p-values are identified for these regions. The potential problem with this method is that it requires a fixed window size. It further creates problems in situations where nucleotide substitution rates vary widely along the length of the sequences. Therefore, the information content of different windows will vary greatly. In the regions of alignment with a lot of variability, small recombinant regions are detected easily compared to portions of alignment with low variability.

5.3.1.4. MAXCHI method

In the MAXCHI method, for every possible sequence pair in the alignment, a window of fixed length with a partition at its center is moved along the sequences one nucleotide at a time (Maynard Smith J., 1992). At each window position in the sequences, a 2x2 chi-

squared value is calculated as an expression of the difference between the pairs of sequences on either side of the central partition of the window. The peaks in the chi-squared values are obvious in the plot, indicating a potential recombinant. This method is one of the most accurate for detecting breakpoints. The potential problem with this method is that it gives high false negatives for both highly divergent and very similar aligned sequences.

5.3.1.5. CHIMERA (Posada and Crandall, 2001)

This method is a modification of the MAXCHI method. The difference between the methods lies in the selection of polymorphic sites. The CHIMERA method uses only triplets of sequences for screening. All monomorphic sites and sites where neither of the two parental sequences matches the recombinant are discarded. Then three sequences are compressed into a linear string of 1's and 0's, where 1 means a match of the recombinant with one parent and 0 means a match with the other parent. A window of fixed length with a partition at its center is moved along the string, one position at a time. At each window position, a 2x2 chi-squared value is calculated as an expression of the difference in the proportion of 1's and 0's on either side of the partition. The chi-squared values are plotted along the length of the alignment and peaks recognize potential recombinants. It is another accurate method for detecting breakpoints. Because MAXCHI and CHIMERA are quite similar to each other, the results should not be compared between these two methods.

5.3.1.6. SISCAN method (Gibbs *et al*., 2000)

In this method, a fourth sequence is constructed either by aligning all possible triplets or by horizontal randomization of one of the sequences in the triplet. It is either the most

divergent sequence in the alignment or the sequence that is most closely related to the three sequences in a triplet, but is more distantly related to the three sequences than they are to one another (i.e. it is the nearest outlier). A fixed length window is moved along four sequences with a set number of nucleotides at a time. If a randomized sequence (produced by a method called horizontal randomization, which maintains the nucleotide content) is used, a new randomized sequence is produced for every window. Each column of alignment is sorted into one of the 15 different categories. The nucleotides in each column are randomized (by a process called vertical randomization). It produces a defined number of permuted alignments. The number of columns for all 15 categories is determined for each permuted alignment. At every window position, a Z-test is used to determine whether the number of columns in that window corresponds to any of the 15 categories that differ significantly from those determined for the vertically randomized alignments.

5.3.1.7. 3SEQ method (Boni *et al.*, 2007)

In this method, all monomorphic sites, and sites where a potential recombinant matches with potential parents, are discarded. All three sequences are then compressed into a string with +1's and -1's; +1 means a match with one parent and -1 means a match with the other. Starting at each end of the +1 and -1 sequence, a running total of the -1's and +1's is recorded at every new position. The maximum difference in the running total across any two sites and the distance between sites is recorded. Whereas the sites bounding the maximum change in the running total indicate the most probable positions of recombination breakpoints. The difference between the running totals recorded at the sites and the number of nucleotides separating them is used to calculate the p-value. The

advantage of this method is that it does not require user defined analysis settings. This method is quite similar to CHIMERA, so any recombinant events detected only by these two methods are considered less reliable.

(The reader is directed to the following references for further details on these methods: 3Seq manual; Boni *et al*., 2007; Gibbs *et al*., 2000; J. Marnard Smith, 1992; Martin *et al*., 2010; Martin *et al*., 2005; Padidam *et al*., 1999; Posada and Crandall, 2001; RDP3 manual)

5.3.2. PHI (pair-wise homoplasy index) test of recombination (implemented in SplitsTree4 software)

In phylogenetic studies, homoplasy is defined as a trait (genetic or morphological) that is shared by two or more taxa because of convergent evolution (Chirat *et al*., 2013). This test determines the recombination event(s) in a set of aligned sequences regardless of population history, geographical location, recombination or mutation rate, and thus minimizes false positives. The test is well suited for the populations with no random mating because it can distinguish between recurrent mutations and recombination events unlike other recombination tests (Bruen *et al*., 2006). The 'PHI statistic (Φ_w)' is based on the notion of refined incompatibility. Two sites will be called incompatible if there is no genealogical history that can be inferred parsimoniously that involves recurrent or convergent mutations (or homoplasies). In traditional incompatibility for a pair of sites, more than one homoplasies are inferred, whereas in actual fact it is only one homoplasy event. The refined incompatibility score overcomes the limitation of compatibility. There are two interpretations of this refined incompatibilty score, one is the absence of recombination, which represents the number of homoplasy events that have occurred in

the population; the other interpretation is that there is no recurrent or convergent evolution in the population; it represents the number of recombination events. The 'PHI statistic' is calculated as:

$$\Phi_w = \frac{2}{k(2n-k-1)} \sum_{j=1}^{k} \sum_{i=1}^{n-j} i(\chi_i, \chi_{i+j})$$

Here, I and j are two sites in the reference, k(2n-k-1) is the normalizing factor.

5.4. Materials and methods

5.4.1. Collection and preparation of *Pst* isolates

A total of 48 uredinial isolates (Table 5.1) of *Pst* were collected between 1984-2013 from western Canada and the USA: 25 from Saskatchewan, 15 from Alberta, five from Manitoba, one from British Columbia, and two from Arkansas. Four of these isolates were collected prior to 2000 (old) and 44 collected after 2000 (new). Except for one isolate collected from foxtail barley (*Hordeum jubatum*), all other isolates were collected from winter or spring wheat. Two isolates were collected in 1984, one in 1990, one in 1997, four in 2005, one in 2007, three in 2010, 18 in 2011, three in 2012 and 15 in 2013.

Table 5.1. Collection information on *Puccinia striiformis* f. sp. *tritici* isolates.

Isolate No. (No. for STRUCTURE output Fig 4.5)	Isolate Name	Year of Collection	Region/Province	Old/New| West/East[a]
1. (17)	W088	1984	Alberta	Old|West
2. (30)	W047	1984	British Columbia	Old|West
3. (14)	AR-90-01	1990	Arkansas, USA	Old|East
4. (15)	AR-97-01	1997	Arkansas, USA	Old|East

5. (38)	W021	2005	Manitoba	New|East
6. (39)	W023	2005	Manitoba	New|East
7. (40)	W024	2005	Manitoba	New|East
8. (41)	W025	2005	Manitoba	New|East
9. (16)	W058	2007	Alberta	New|West
10. (31)	W055	2010	Alberta	New|West
11. (34)	W015	2010	Alberta	New|West
12. (35)	W017	2010	Alberta	New|West
13. (36)	W018	2011	Alberta	New|West
14. (45)	W054	2011	Alberta	New|West
15. (42)	W028	2011	Manitoba	New|East
16. (32)	W031	2011	Alberta	New|West
17. (48)	W034	2011	Alberta	New|West
18. (33)	W056	2011	Alberta	New|West
19. (1)	W001	2011	Saskatchewan	New|East
20. (2)	W002	2011	Saskatchewan	New|East
21. (3)	W003	2011	Saskatchewan	New|East
22. (4)	W004	2011	Saskatchewan	New|East
23. (5)	W005	2011	Saskatchewan	New|East
24. (6)	W006	2011	Saskatchewan	New|East
25. (7)	W007	2011	Saskatchewan	New|East
26. (8)	W008	2011	Saskatchewan	New|East
27. (9)	W009	2011	Saskatchewan	New|East
28. (10)	W010	2011	Saskatchewan	New|East
29. (11)	W011	2011	Saskatchewan	New|East
30. (12)	W012	2011	Saskatchewan	New|East
31. (20)	W030	2012	Saskatchewan	New|East
32. (24)	W052	2012	Alberta	New|West
33. (25)	W053	2012	Alberta	New|West
34. (23)	W013	2013	Saskatchewan	New|East
35. (37)	W019	2013	Saskatchewan	New|East
36. (27)	W029	2013	Saskatchewan	New|East
37. (18)	W037	2013	Saskatchewan	New|East
38. (19)	W038	2013	Saskatchewan	New|East
39. (26)	W039	2013	Saskatchewan	New|East
40. (21)	W040	2013	Saskatchewan	New|East
41. (13)	W042	2013	Saskatchewan	New|East
42. (47)	W043	2013	Saskatchewan	New|East
43. (22)	W044	2013	Saskatchewan	New|East
44. (29)	W045	2013	Saskatchewan	New|East
45. (28)	W046	2013	Saskatchewan	New|East
46. (43)	W049	2013	Alberta	New|West
47. (44)	W051	2013	Alberta	New|West
48. (46)	W060	2013	Alberta	New|West

[a] Old= collected before 2000; New= collected after 2000; West= BC or AB; East= SK or MB

'Avocet -*YrA*' wheat, which is susceptible to all known races of *Pst* in North America, was used to increase urediniospores. Seedlings at the two-leaf stage were inoculated with *Pst* collected from the infected leaves. The inoculated plants were kept in a dew chamber at 10˚C for 48 hours in the dark and then transferred to a growth chamber with a diurnal temperature cycle that gradually changed from 12˚C to 17˚C, with a 16 hour photoperiod and 60% relative humidity. Plants inoculated with each sample were covered with plastic to prevent cross-contamination of the samples. Generally, 14 days post-inoculation (dpi), pustules appeared on leaves. The raw samples collected from fields were processed to obtain single pustule isolates by transferring a single pustule with a very fine needle to another seedling. This cycle was repeated a number of times to increase the amount of spores required. The urediniospores were collected by tapping infected leaves over aluminum foil. The spores collected were cleaned to remove any dust particles or plant tissue and the clean spores were put into eppendorf tubes. Spores were desiccated using silica at 4˚C for at least three days and then stored at -80˚C.

The virulence phenotypes of the isolates were already characterized using the methods described in Chapter 4. The two isolates from Arkansas, USA were already characterized as race PST-3 (Milus *et al*., 2006).

5.4.2. Genomic DNA extraction and quantification

Genomic DNA was extracted from each isolate/sample using following procedure. Urediniospores (10 mg) were mixed with 25 mg of diatomaceous earth, 1 g of 0.8 mm diameter zircon spheres plus one 2 mm zircon sphere in a 2 ml microcentrifuge tube. The sample was processed for 10 seconds in a FastPrep FP120 machine at setting no. 5. The

samples were processed again for an additional 10 seconds (BioTechniques, 2002). The rest of the extraction was done using the modified cetyltrimethylammonium bromide (CTAB) procedure (Chen *et al*., 1993; Liu *et al*., 2012). The crushed spores were mixed with 500 µL of extraction buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl and 100 mM EDTA). After adding 30 µL 20% SDS, 75 µL 5M NaCl and 65 µL CTAB/NaCl, and mixing thoroughly, the tubes were incubated at 65^0C for 60 min. The mixture was extracted with an equal volume of saturated phenol (phenol/chloroform/ isoamyl alcohol 25:24:1) and a 0.1 volume of 3 M sodium acetate (pH 5.3), and was centrifuged for 10 min at 13,000 rpm. The top aqueous phase was transferred to a clean tube. After adding an equal volume of chloroform, the tubes were gently inverted and centrifuged for 10 min at 13,000 rpm. The DNA was precipitated by adding an equal volume of isopropyl alcohol and a 0.1 volume of 3 M sodium acetate (pH 5.3) at -20^0C for 120 min. After centrifuging for 30 min at 4^0C, the pellet was rinsed twice with cold 70% ethanol and 100% ethanol separately, dried and dissolved in 500 µL TE buffer. The DNA solution was treated with RNase (final concentration 10 µg/mL) and kept at 37^0C for 60 min to completely digest the RNA. The DNA was re-precipitated, rinsed with ethanol, dried and dissolved in 30 µL of TE buffer. DNA concentrations were diluted to 30 ng/µL with TE buffer before storing in small aliquots at -20^0C (Chen *et al*., 1993; Liu *et al*., 2012). DNA was quantified using PicoGreen® (Molecular Probes/Invitrogen Eugene, OR 97402) and DNA concentrations were normalized to 10 ng/µl.

5.4.3. Library preparation and illumina HiSeq2500 sequencing

The genomic DNA library was prepared for each sample using the TruSeq Nano DNA sample preparation kit from Illumina. For each library, 100 ng starting DNA was used and the protocol for 350 base pair inserts was followed. The quality of each completed library was assessed using a DNA 1000 chip on the 2100 Bioanalyzer (Agilent Technologies Inc., UK). The concentration of each library was determined by qPCR using the KAPA SYBR FAST ABI Prism qPCR Kit (Kapa Biosystems) with the StepOnePlus Real-Time PCR system (Applied Biosystems). All libraries were then diluted to 2 nM. Equimolar concentrations of 24 libraries were pooled and a final concentration of 12 pM was used for clustering each pool on two lanes of a Rapid PE flowcell. The samples were sequenced (2 x 101 cycles, paired-end reads) on the HiSeq2500 (illumina) using the TruSeq Rapid SBS Kit-HS 200 cycles (Illumina), in the DNA Technologies Laboratory of the National Research Council of Canada, Saskatoon, Saskatchewan, Canada.

5.4.4. Variant calling

Illumina HiSeq2500 paired-end reads for each isolate were aligned to the *Pst* race 'PST-78' reference genome (the Broad Institute, 2014, http://www.broadinstitute.org) using Novoalign version 2.05.20 (http://www.novocraft.com) for variant calling. Sequence alignment map (SAM) format files were produced by Novoalign and SAMtools (Li *et al*., 2009) and run onto SAM files to create sorted BAM files. Reads with <51 bp length and quality value <20 were discarded. The reference genome of 'PST-78' is from both nuclei of dikaryotic urediospore and is a hybrid of both nuclei, which is the same as a diploid (Les Szabo, personal communication).

5.4.5. Group assignment and tests of genetic differentiation based on genetic distance

The variant calling format file (VCF) was obtained after analyses of the raw reads generated by HiSeq2500 sequencing. The genome-wide SNPs discovered after mapping against the reference genome numbered approximately 1 million (0.85% polymorphism in the genome). The raw SNPs were quality filtered for good quality reads (QUAL.) and depth (DP). The parameters set for quality filtering and the corresponding numbers of SNPs obtained are showed in Table 5.3.

The genetic relationship of the 48 *Pst* isolates were determined using 4,510 high quality SNPs (>50X coverage) with four different approaches. The first was to generate a neighbor-joining split-network by using SplitsTree4 (Huson and Bryant, 2006) based on a dissimilarity matrix of the high quality SNPs. The second was to generate NJ-tree on the best selected model by using MEGA v. 6.06 (Tamura *et al*., 2013) based on high quality SNPs. Bootstrap re-sampling (1000 replications) was used to measure the reliability of individual nodes in each phylogenetic tree. A discrete Gamma distribution was used for NJ-tree to model the evolutionary rate differences among sites (5 categories (+G, parameter)).

The third approach was to perform principal component analysis (PCA) to infer relationships among the 48 isolates using the Unscrambler®X ver. 10.3 (CAMO Software, Norway). Fourth, the program STRUCTURE v. 2.3.4 (Pritchard *et al*., 2000) was used to analyze the population structure of *Pst*. This program used the Bayesian model-based clustering method to identify the number of sub-populations (*K*) present in the western Canadian population of *Pst*. To do this, the admixture model was used, which takes into

account independent allele frequencies among sub-populations with no apriori identification of clustering pattern or geographical location of individuals. A burnin of 35,000 Markov chain Monte Carlo (MCMC) iterations and a run of 50,000 MCMC iterations was used with *K* set as 1-8 with five repetitions. The value of *K* best supported by the model was determined using the method described by Evanno *et al*. (2005), implemented in the program STRUCTURE HARVESTER (Earl and von Holdt, 2012). The STRUCTURE software was capable of identifying the population structure of *Pst*, although it assumes random mating and linkage equilibrium within populations, which is rare in *Pst*.

5.4.6. Recombination analyses

We used the recombination detection program v.4.38 (RDP4) to identify unique recombination events (Martin *et al*., 2010). The RDP4 uses multiple parameters to detect recombination and examines isolates in sets of three. The RDP4 identifies only unique recombination events, i.e. it ignores the same recombination event in closely related individuals. All recombination analyses were done using default settings in RDP4, except for the reference option set as 'internal reference only'. Recombination events were considered significantly more likely than mutations if they were detected by two or more analyses in RDP4. Because of the similarity in the detection of recombinants, the CHIMERA method (implemented in RDP4) is quite similar to the MAXCHI and 3Seq methods (implemented in RDP4) (RDP3 manual). The recombinants detected by all these methods were not considered reliable, unless accompanied by some other method. Because of the large number of recombination events (see results), we did not try to identify putative parents and recombinants.

Genome-wide SNPs were used in RDP4 as an input because RDP4 uses only polymorphic sites to detect recombination. High quality SNPs (>50X) were used for recombination tests to avoid false calls for recombinants. Therefore, we used the SNP data with the rationale that SNPs ordered according to their positions on supercontigs in the PST-78 sequenced reference genome are a proxy for actual sequence data. The 'PHI-test' implemented in the software SplitsTree4 was also used to test recombination within clades.

5.5. Results

5.5.1. Whole genome re-sequencing

To generate a SNP resource for genome analysis of *Pst*, we performed shotgun sequencing, the whole genome next-generation sequencing (NGS) on 48 *Pst* isolates. On average, 12,711,460 reads of each sample were generated by the illumina HiSeq2500 platform (Table 5.2). The average genome coverage was 11X with the greatest coverage of 24X for isolate W009 and the lowest, 7X for isolate AR-97-01 (PST-3) (Table 5.2).

Table 5.2. Number of reads obtained and genome coverage for each *Pst* isolate.

Isolate No.	Isolate Name	NOR[a]	Genome Coverage[b]
1.	W088	11,634,891	10X
2.	W047	14,614,936	13X
3.	AR90-01 (PST-3)	11,266,641	10X
4.	AR97-01 (PST-3)	8,512,705	7X
5.	W021	13,052,024	11X
6.	W023	12,299,476	11X
7.	W024	12,085,319	10X
8.	W025	11,455,038	10X
9.	W058	18,637,936	16X
10.	W055	14,333,501	12X
11.	W015	11,766,452	10X
12.	W017	9,878,518	8X

13.	W018	11,982,773	10X
14.	W054	22,353,931	19X
15.	W028	13,282,607	11X
16.	W031	12,173,614	10X
17.	W034	13,227,186	11X
18.	W056	11,863,098	10X
19.	W001	12,971,415	11X
20.	W002	12,902,393	11X
21.	W003	14,070,381	12X
22.	W004	10,099,439	9X
23.	W005	13,618,331	12X
24.	W006	16,219,684	14X
25.	W007	14,093,847	12X
26.	W008	15,493,370	13X
27.	W009	28,235,955	24X
28.	W010	11,619,907	10X
29.	W011	12,958,491	11X
30.	W012	14,919,672	13X
31.	W030	13,053,778	11X
32.	W052	9,329,236	8X
33.	W053	11,029,289	9X
34.	W013	12,406,001	11X
35.	W019	10,414,732	9X
36.	W029	14,375,044	12X
37.	W037	14,551,039	13X
38.	W038	9,881,670	8X
39.	W039	15,097,675	13X
40.	W040	12,588,642	11X
41.	W042	12,324,726	11X
42.	W043	10,588,242	9X
43.	W044	12,930,482	11X
44.	W045	13,885,800	12X
45.	W046	16,537,875	14X
46.	W049	12,905,974	11X
47.	W051	9,654,536	8X
48.	W060	14,318,235	12X
Average	**--------**	**12,711,460**	**11X**

[a]Number of reads generated from HiSeq2500 sequnecing; [b]Coverage=(Length of read*NOR)/Genome size; Genome size=117 Mb (the Broad Institute, 2014)

5.5.2. SNP discovery

Mapping of reads from the 48 *Pst* isolates against the reference genome of race PST-78 generated approximately 1 million SNPs. This indicates an abundance of SNPs in the *Pst* genome, with an average of one SNP every 117 bp. After quality filtering, the maximum number of good SNPs with >10X coverage were 247,948 (0.2% genome-wide ploymorphic sites) (Table 5.2). To have confidence in a SNP call, SNPs with coverage of >50X were used for downstream analyses.

For each SNP (>10X coverage), the directionality of the change can not be inferred from the data, thus polymorphisms were grouped alphabetically, that is, A>G and G>A were grouped as A>G and so on (Appendix-II). A greater number of transitions (A>G or C>T) (193,781) than transversions (A>C, A>T, C>G or G>T) (86,372) were identified. The average ratio of transition to transversion was 2.5:1 (Appendix-II).

A large number of heterozygous SNPs were detected in the *Pst* genome for all 48 isolates. The maximum percentage of heterozygous SNPs was 58.8% for isolate W052 and the miminum was 28.6% for isolate W052. The average number of heterozygous SNPs in the *Pst* genome numbered 133,760 (47.7%) (Appendix-III).

Table 5.3. Number of SNPs corresponding to different quality parameters set for filtering raw SNPs.

Quality parameters		Number of SNPs obtained
Quality value of the read (QUAL.)	**Depth of base/SNP (DP)[a]**	
>20	>10X	247,948
>20	>20X	27,777
>20	>30X	13,010
>20	>40X	7,312
>20	>50X	4,510

[a]every read having SNP with coverage less than mentioned in the table was rejected.

5.5.3. Genetic differentiation and population structure of *Pst*

The genetic relationship of the 48 *Pst* isolates were illustrated in neighbor net, NJ-tree and STRUCTURE output in Figures 5.1, 5.3 and 5.4. Different diversity analyses divided the *Pst* population from western Canada into 4 sub-populations (Figures 5.1-5.4). It is clear by the clustering pattern of isolates that the old isolates (collected before 2000) are genetically different from new isolates (collected after 2000), because 3 of the 4 old isolates comprise one group (G2-Red label) along with some new isolates. Also, the two isolates from Arkansas, USA grouped together and are different from most of the Canadian isolates (Figures 5.1 and 5.3). A few reticulated topologies from the neighbor-net indicated some signs of potential recombination events (Huson and Bryant, 2006). In addition, isolates W029 and W056 (G3-Green label), grouped together and are quite different from all other isolates. Isolate W029 was sampled from foxtail barley (*Hordeum jubatum*), which may serve as an accessory host plant for the *Pst* fungus, although W056 was sampled from common wheat (*T. aestivum*). Isolates W011, W053, W028 and W045 share some similarity among groups G1 and G4 on the both sides of the neighbor net (Fig. 5.1). This similarity is

also evident in the results produced by STRUCTURE (Fig. 5.4, Table 5.1). The NJ-tree (Figure 5.3) grouped two isolates, W029 and W056, along with some other isolates; however, it was considered a separate clade based on results from STRUCTURE (Figure 5.4) and neigbor-net tree produced by SplitsTree4 (Figure 5.1).

In relation to geographical sampling location of isolates (i.e. western praires comrising BC and AB or eastern prairies comprising SK and MB), the analyses didn't indicate any significant differentiation of the *Pst* population between the regions (Table 5.1, Figures 5.1-5.4). The only weak relation was that most of the isolates collected from the western prairies (AB or BC) were grouped in groups G2 and G3. In Clade 3, one isolate from AB is grouped with the isolate sampled from foxtail barley (*Hordeum jubatum*) and the two isolates grouped together. Group G1 consisted mainly of isolates collected in or before 2011, and group G3 consisted mostly the isolates collected in 2013 (Table 5.1, Fig. 5.3). There is quite a difference in virulence spectrum among isolates in these two groups, the latter with a relatively wider virulence spectrum (refer to CHAPTER 4).

The Bayesian clustering model implemented in STRUCTURE indicated that K=4 was the optimal grouping of the stripe rust isolates/individuals. The number of groups/clusters suggested by STRUCTURE were similar to the groups formed by different dendrograms. However, STRUCTURE output results indicated the proportion of each individual isolate in every cluster/group (Figure 5.4). The STRUCTURE program was able to distinguish all 4 groups clearly (Figures 5.1-5.3). When K=2 was chosen, the STRUCTURE formed two groups, one with *Pst* isolates AR-90-1 (Old), AR-97-01 (Old), W018, W058, W052, W060,

W047(Old), W015, W029 (sampled from foxtail barley), and W056 and the other with the rest of the isolates.

The first three principal components from the PCA explained 23% (PC1-10%, PC2-9%, and PC3-4%) of the variation in the data. The tight clustering of most of the isolates in the PCA plot indicated the presence of a few wide-spread sub-populations on the Canadian prairies and little diversity in the population (Figure 5.2). In the PCA plot, isolates W029 & W056 and groups formed by neighbor net and NJ-tree (Figures 5.1 and 5.3) are well separated from each other.

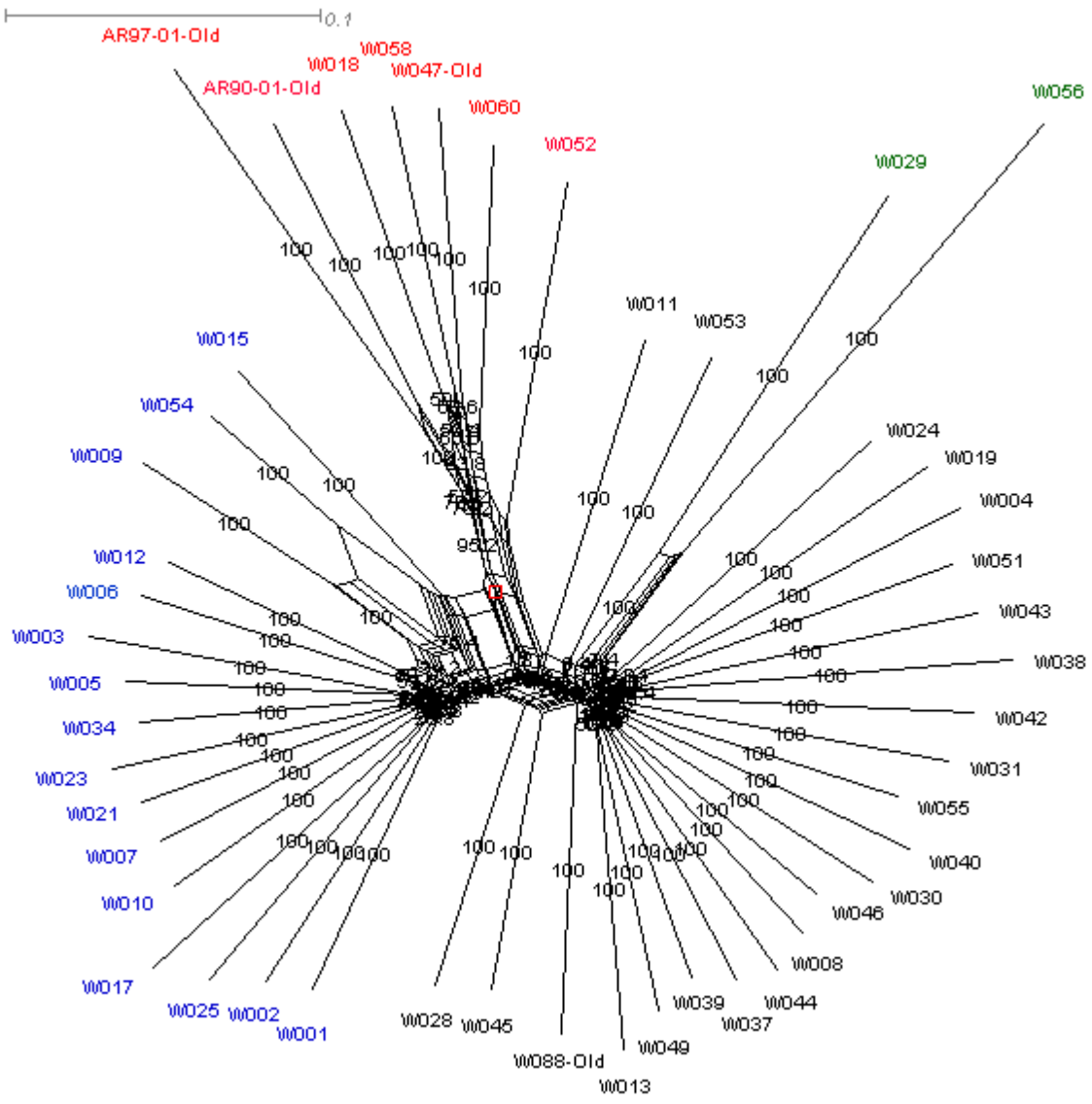


Figure 5.1. Neighbor-joining network created using uncorrected-P characters transformation, showing the genetic relationship of 48 *Puccinia striiformis* f. sp*. tritici* isolates, obtained by using SplitsTree4 software. Isolates with the same label color belong to the same group/sub-population. In figure, groups are labelled with colors: G1-Blue, G2-Red, G3-Green, G4-Black.

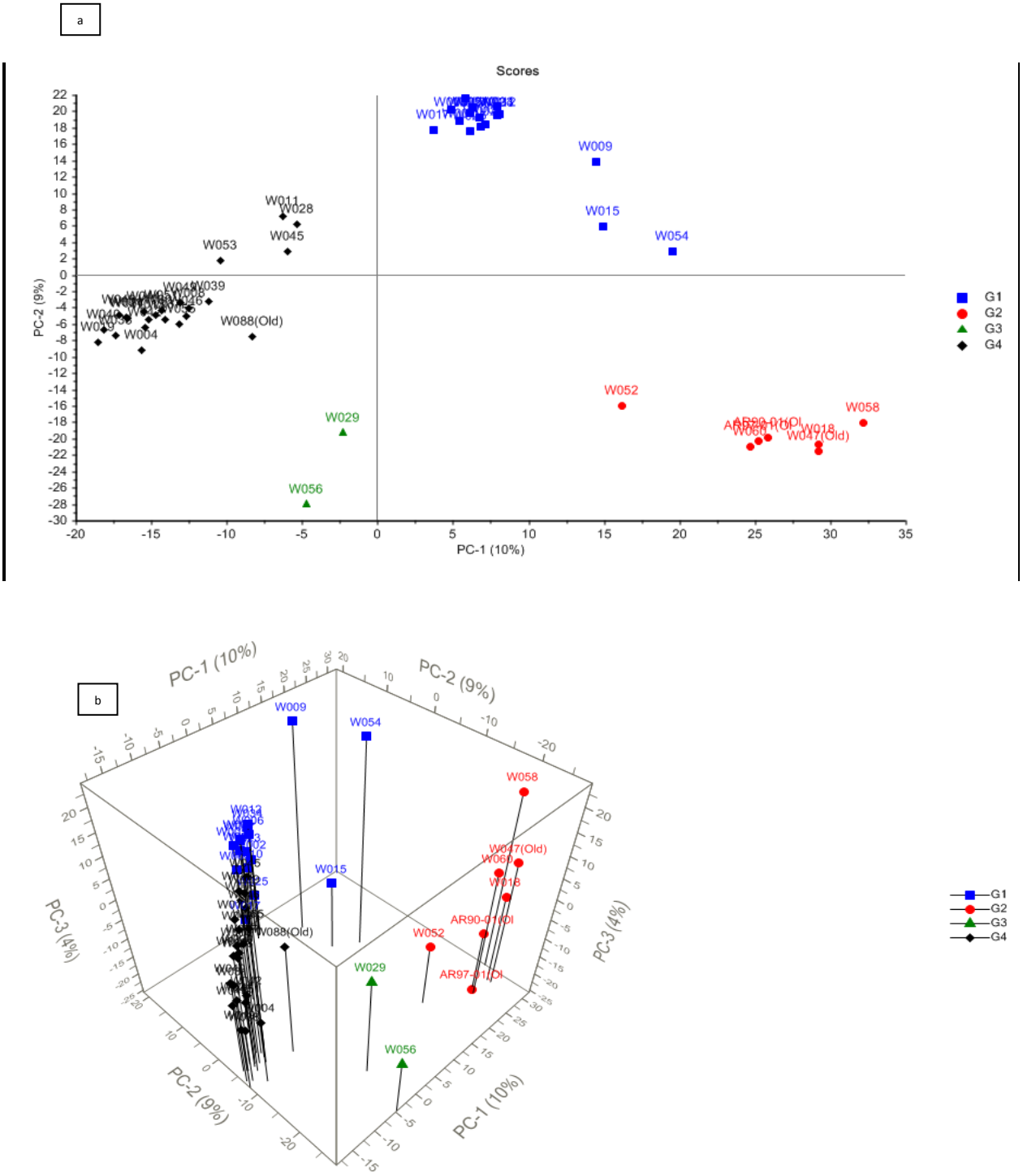


Figure 5.2. Single nucleotide polymorphism (SNP)-based (SNP coverage >50X) genetic relationship of 48 *Puccinia striiformis* f. sp. *tritici* inferred with the principal component analysis (PCA) generated by the Unscramber®X software (ver. 10.3) (a) 2-D plot with PC-1 and PC-2, and (b) 3-D plot with the first three principal components.

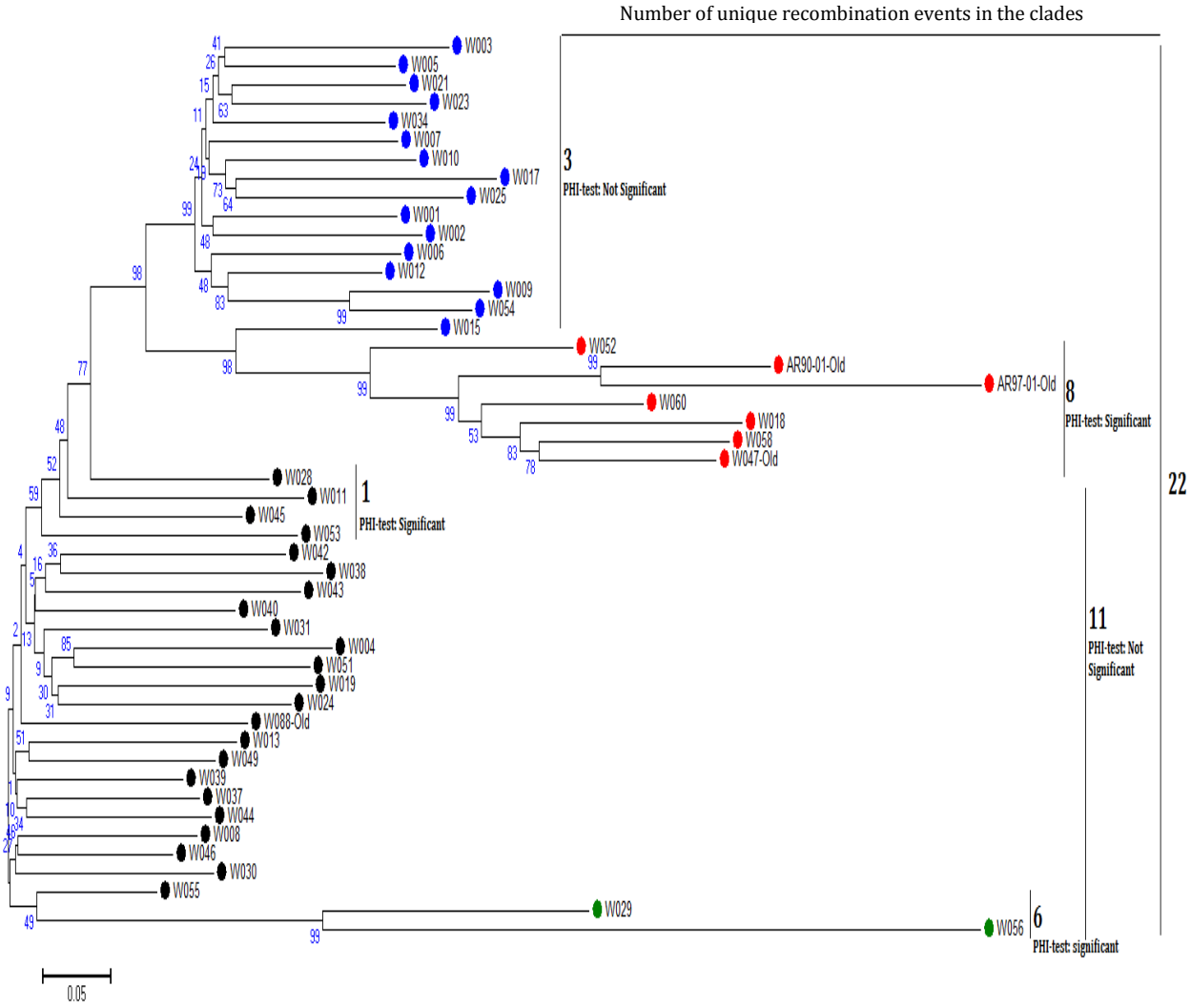


Figure 5.3. NJ-tree (bootstrap=1000) based on a similarity matrix of 48 *Puccinia striiformis* f. sp. *tritici (Pst)* isolates generated by MEGA6 software (ver. 6.06) indicating the genetic relationship among isolates and the distribution of recombination events in *Pst.* The number of unique recombination events detected by RDP4 v.4.38 associated with each hierarchy in the tree is shown on the right (see also Table 5.3). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (0.02). In the tree, isolates with the same color code belong to the same clade/group/sub-population.

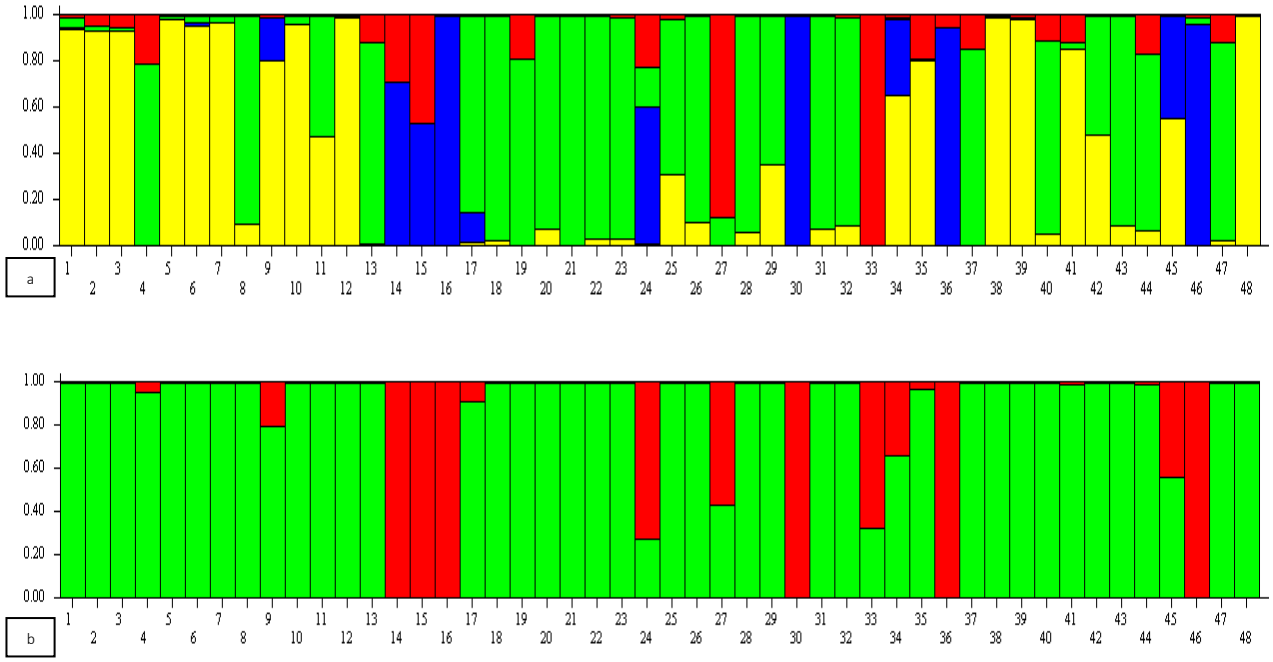


Figure 5.4. The population structure of *Pst* inferred by model-based Bayesian cluster analysis of genome-wide SNP data (calculated by STRUCTURE). Results indicate: (a) the optimum number of clusters (*K*=4) detected by the software (b) population division into two clusters (*K=2*). On the X-axis of the graph, the numbers represent the isolate number (Table 5.1) and the Y-axis is the proportion of SNPs in each isolate in every cluster.

5.5.4. Tests for recombination

The different methods implied that four-subpopulations of *Pst* are prevalent in western Canada. Corresponding to these four sub-populations/clades in Figure 5.3, recombination tests detected 3 unique events in Clade 1, 8 in Clade 2, 11 in Clades 3 & 4 and only 6 in Clade 4 (Table 5.4). One unique recombination event was detected among the isolates W028, W011, W045 and W053, which share a genetic identity between Clade 1 (Figures 5.3, 5.4, Table 5.1). These four isolates were tested for recombination because their

positions in between two well defined clades in the neighbor net (Fig. 5.1) and NJ-tree (Fig. 5.3) suggested that they could be recombinant.

In Clade 3, after including W029 and W056, we detected 11 unique recombination events and 6 events in Clade 4 (along with isolate W088), which means of 11 events, 6 could be attributed solely to isolates W029, W056 and W088. From these analyses, we speculate that recombination occurred in some individuals of the *Pst* population. In relation to the number of individuals in each clade, the maximum numbers of recombination events were detected in Clade 2 and Clade 4. The isolates covered in recombination events have a weak virulence combination from their parents (data not showed).

The 'PHI-test' implemented in SplitsTree4 software, found significant evidence of recombination in Clades 2 and 4 and in the group of isolates: W028, W053, W011 and W045 (Figure 5.3, Table 5.4).

Table 5.3. Recombination detected within clades using program RDP4 v. 4.38.

Group/Clade	**Number of individuals in clade**	**Number of recombination events detected[a]**	**PHI-test (p-value)**
1	15	3	0.1257
2	8	8	6.437×10^{-5}
3 & 4	23	11	0.2657
3	2	6[b]	2.139×10^{-7}

[a]Number of recombination events was detected using default parameters for significant events in the RDP4 program; [b] A minimum of three samples were required for testing recombination in Phi-test and RDP4 prog., thus an additional closely related isolate was included along with the two isolates.

5.6. Discussion

The aim of this study was to differentiate populations from the western prairies (AB and BC) from the eastern prairies (SK and MB) by examining the genetic variation in the population of *Pst* in western Canada. The population of *Pst* in western Canada is dominated by a few widespread sub-populations. The inability to distinguish between populations from the western and the eastern prairies could be attributed to the lack of any geographical barrier. Moreover, air-borne and long-distance dispersal of *Pst* by wind could have resulted in inter-mixing of races from the west with races from the east. Based on the virulence spectrum, the Canadian isolates collected in 2011, which have a narrow virulence spectrum, are differentiated from isolates collected in 2013, which have a wider virulence spectrum (Brar and Kutcher, unpublished data). This could be attributed to the change in genomic regions governing virulence of the 2013 population by forces such as mutation and selection pressure.

Signs of recombination were observed in the *Pst* population. The maximum number of recombination events was detected in only a few individuals of the population, and most of these individuals are grouped in one or two sub-populations, suggesting that the population of *Pst* in western Canada is largely clonal (Holtz *et al*., 2013), although there is indication of possible recombination. The results for recombination by the 'PHI-test' implemented in SplitsTree4, are more reliable than methods implemented in RDP4 because the 'PHI-test' is able to distinguish between recurrent mutations from recombination events (Bruen *et al*., 2006). This could be the reason for the fewer recombination events detected by RDP4 relative to the 'PHI-test'.

The discovery of the alternate host in 2010 by Jin *et al*. (2010) may explain the high diversity of *Pst* populations in China and Asia (Zheng *et al*., 2013, Ali *et al*., 2014), however, there is no report of isolation of *Pst* from an alternate host in North America (Wang *et al*., 2013; Anmin Wan, personal communication). Although populations do not appear to be derived from sexual recombination, it does not mean it is not present. In fact the other way around, it is possible that the potential sexual population is not frequent enough to be sampled from wheat fields. One of the alternate hosts, *Mahonia aquefolim* L. (Oregon Grape) is widely used as an ornamental plant in California and some areas of the PNW of the USA (Wang *et al*., 2013). The population in the western Canadian prairies arrives via winds coming from the PNW (Kumar *et al*., 2012; Su *et al*., 2003) and thus detection of recombination in the western Canadian population should be possible. Another possible explanation for detection of recombination in *Pst* could be attributed to somatic recombination, which is one of the mechanisms that cause variation in *Pst* populations (Chen, 2005; Hovmøller et al., 2011). Somatic hybridization is reported in *Pst* in empirical studies (Little and Manners, 1969; Goddard, 1976; Wright and Lennard, 1980). A recent study by Wang and McCallum (2009) suggested the possibility of spore somatic recombination and virulence recombination in the closely related rust fungus, *Puccinia triticina,* which may explain recombination events in *P. striiformis*. Also, the occurrence of races in nature, resulting from somatic hybridization in *Puccinia graminis* f. sp. *tritici*, was reported (Burdon *et al*., 1981).

Somatic recombination in *Pst* can be determined from virulence recombination (in recombinant races) from co-existing races (potential parents). Some of the isolates in the present study showed some signs of virulence combinations from co-existing isolates, but

the lack of strong evidence could be attributed to the few isolates that were sequenced from the same county or area. Also, in highly clonal populations, STRUCTURE software is unable to characterize the population (Halkett *et al*., 2005), but in the present study results from STRUCTURE are similar to other analyses, indicating the partial clonal nature of *Pst.* STRUCTURE clearly differentiated individuals showing signs of recombination from others when the population was divided into two groups, which further supports some lineages derived by somatic or sexual recombination. The highly heterozygous nature of *Pst* may be attributed to difference in the two nuclei of the urediniospores of *Pst* (Zheng *et al*., 2014; Les Szabo, personal communication).

5.7. Conclusions

The results from the present study suggested that the *Pst* population in western Canada is not solely clonal as some somatic or even sexual recombination events might be a possibility. The diversity of *Pst* in Canada is low compared with other countries such as China and Pakistan where sexual recombination is known to occur (Ali *et al*., 2013; Zheng *et al*., 2014). The recombinant population in western Canada is distinguishable from the clonal population. The partial clonal nature of *Pst* in western Canada indicates the need for more studies on somatic recombination events so that the population structure, in terms of virulence, can be better understood.

CHAPTER 6. SYNTHESIS AND CONCLUSIONS

The fungus *Puccinia striiformis* f. sp. *tritici* is the causal agent of stripe rust disease in wheat and is of worldwide occurrence (Chen, 2005; Xi *et al*., 2014). *Puccinia striiformis* f. sp. *tritici* became a major pest of wheat in western Canada in the past few years and stripe rust epidemics in 2005, 2006 and 2011 in AB and SK highlighted the importance of management (Kutcher *et al*., 2012; McCallum *et al*., 2006; Xi *et al*., 2014). Successful management of this disease and development of resistant varieties demands knowledge of virulence spectra of the pathogen population in all regions. The pathogen population shifts rapidly in terms of virulent races, depending on selection pressure. Thus characterization of the population every three to four years is necessary. The present work is a detailed study of variation in the *Pst* population in western Canada.

6.2. Relationship of virulence phenotypes and molecular genotypes

This thesis explained the virulence and genetic variation detected in the *Pst* population in western Canada. The population was divided into four sub-populations in terms of virulence (Chapter 4) and genotypic variation (Chapter 5). There was no relationship observed among these sub-populations detected in the two studies. Also, in these two different studies, isolates closely related to each other in terms of virulence were different genotypes. Genotypic variation was independent of virulence variation in all analyses. The Mantel test, implemented in GenAlex version 6.5 (Peakall and Mouse, 2012) was done to test the association between virulence and genetic distance among 48 *Pst* isolates, which resulted in an insignificant association (P >0.05).

Previous studies on genotypic variation in this pathogen population did not detect any relationship with virulence phenotypes or races (Chen, 2005; Chen *et al*., 1995). This may be explained by the fact that virulence in a *Pst* isolate or individual is governed by only a few loci or genes (Markell and Milus, 2008), thus variation in these loci or genomic regions can explain the relationship of different races.

6.3. Implications and future research

The *Pst* population prevalent in the prairie provinces of Canada is very diverse in terms of virulence. Except for a few genes, virulence was detected in the pathogen population for all others. Sixty-one isolates of *Pst* collected from western Canada from 1984-2013 were divided into 33 races. Except for three races (C-PST-1, C-PST-2, C-PST-3), all other races were represented by one or two isolates. The frequent detection of race C-PST-1 from the prairies explains the parasitic fitness or natural selection of the race. Except for a few races, most of the races are different from each other in terms of virulence/avirulence on a single gene. This explains the step-wise addition of virulence in the pathogen races. But some closely related races were also different from each other by two or three virulence factors, which suggest some other mechanism of variation in addition to single point mutations.

Most Canadian wheat varieties are deployed with *Yr18* or *Yr36*, which are APR genes. These APR genes are expressed only at the adult plant stage and do not confer resistance to stripe rust races at the seedling stage. Until 10-15 years ago, stripe rust was not a major problem for wheat growers on the prairies as it usually arrives late in the wheat growing season, when crops were near maturity. At that stage, APR genes were successful in mitigation of stripe rust attack. But, recent research has provided evidence for

overwintering of *Pst* in western Canada. Successful overwintering can cause early attack of spring wheat and thus cause yield losses. Epidemics of stripe rust in the years 2005, 2006, 2011 in western Canada caused significant losses. This highlights the importance of developing wheat varieties resistant to stripe rust races at all-stages. Successful management of this disease is possible by cultivation of resistant varieties because host resistance is the most efficient and economical way to manage this major disease of wheat. On the other hand, the virulence spectra of the pathogen population will indicate the sources of resistance that can be successfully utilized by wheat breeders for varietal development against the most common and virulent races of the pathogen.

There is a high level of genetic variation in the western Canadian *Pst* population as detected using genome-wide SNP variants. The genetic data from 48 *Pst* isolates indicated the relationship (although not a strong relationship) of clades, in term of virulence and year of collection. Also, some *Pst* isolates showed signs of recombination. The *Pst* population in North America is considered clonal based on recent research done (Holtz *et al*., 2013). Although the alternate host of *Pst* was discovered in 2010, and another alternate host (*Mahonia aequifolium*) in 2013, but the aecial stage of the fungus was not recovered in North America. Somatic hybridization is another mechanism of variation in cereal rusts, although somatic recombination events are relatively less likely to occur in nature as compared to point mutations and selection pressure. Natural somatic hybrids in closely related rusts were reported in the past (Burdon *et al*., 1981; Chen *et al*., 2010). The detection of somatic recombination events in the present study can not be ignored and it warrants further research to test recombination mechanisms in *Pst*.

To conclude, the *Pst* population has a great potential to change in terms of virulence and genetic make-up. Some mechanisms of genetic variation such as somatic recombination and the parasexual cycle are not well documented in the *Pst* population, which suggests more research is needed to explain the variability in the population.

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Appendix-I. Sampling information, races, and virulence profiles of all *Puccinia striiformis* f. sp. *tritici* isolates examined in this study.

Isolate	Host	Year	Location	Race	Avocet - *YrA*	*YrA*	*Yr1*	*Yr2*	*Yr5*	*Yr6*	*Yr7*	*Yr8*	*Yr9*	*Yr10*	*Yr15*	*Yr17*	*Yr18*	*Yr24*	*Yr26*	*Yr27*	*Yr28*	*Yr29*	*Yr31*	*Yr32*	*YrSP*	*Yr5u*	*Yr3b,4b*	*Yr3a,4a*	*Yr18+36+29*	*Yr2+HVII+25*	*YrTye*	AC Barrie	AC Avonlea	CDC Teal	Chinese 166	Brevis (Triticale)
W011	Wheat	2011	SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W004	Wheat	2011	Hanley, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W041	Wheat	2013	Chaplin, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W061	Wheat	2013	Lethbridge, AB	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W027	Wheat	2013	Canora, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W028	Wheat	2011	Winnipeg, MB	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W045	Wheat	2013	Swift Current, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W088	Wheat	1984	Bow Island, AB	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W037	Wheat	2013	Canora, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W055	Wheat	2010	Calgary, AB	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W024	Wheat	2005	Winnipeg, MB	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W014	Wheat	2013	Yellow Creek, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W042	Wheat	2013	Aberdeen, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W013	Wheat	2013	Melfort, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W046	Wheat	2013	Stornoway, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W038	Wheat	2013	Foam Lake, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W043	Wheat	2013	Wakaw, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W019	Wheat	2013	Landis, SK	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W051	Wheat	2013	Lethbridge County, AB	C-PST-1	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W009	Wheat	2011	Richardson, SK	C-PST-2	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	-	+
W012	Wheat	2011	Balcarres, SK	C-PST-2	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	-	+
W017	Wheat	2010	Lethbridge County, AB	C-PST-2	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	-	+
W053	Wheat	2012	Lethbridge, AB	C-PST-3	+	+	-	+	-	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	+
W050	Wheat	2013	Lethbridge County, AB	C-PST-3	+	+	-	+	-	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	+
W044	Wheat	2013	MacDowall, SK	C-PST-3	+	+	-	+	-	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	+
W022	Wheat	2013	Outlook, SK	C-PST-4	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	-	+
W026	Wheat	2013	Macrorie, SK	C-PST-4	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	-	+
W057	Wheat	2009	Creston, BC	C-PST-5	+	+	-	+	-	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W052	Wheat	2012	Lethbridge County, AB	C-PST-5	+	+	-	+	-	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W020	Wheat	2013	Landis, SK	C-PST-6	+	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	+
W048	Wheat	2013	Fairfield, AB	C-PST-6	+	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	+
W047	Wheat	1984	Creston, BC	C-PST-7	+	+	-	+	-	+	+	-	+	-	-	+	+	-	-	+	+	+	+	+	-	-	-	+	-	-	-	+	+	+	-	-
W054	Wheat	2011	AB	C-PST-7	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	-	-	+	-	-	-	+	+	+	-	-
W029	Foxtail Barley	2013	Smuts, SK	C-PST-8	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	-	-	-	+	+	+	-	-
W030	Wheat	2013	Goodale, SK	C-PST-8	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	-	-	-	+	+	+	-	-
W006	Wheat	2011	Lemsford, SK	C-PST-9	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	+	-	+
W001	Wheat	2011	Whiteshore Lake, SK	C-PST-10	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	+	-	-	-	+	-	+	+	+	-	+
W005	Wheat	2011	St. Brieux, SK	C-PST-11	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	-	+	-	+	+	+	-	+
W021	Wheat	2005	Winnipeg, MB	C-PST-12	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	-	+	-	+	+	+	-	-
W007	Wheat	2011	North Battleford, SK	C-PST-13	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-
W033	Wheat	2011	Paintearth, AB	C-PST-14	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	-	-	-	-	+	-	+	+	+	-	-

W035	Wheat	2011	Lacombe, AB	C-PST-15	+	+	-	+	-	+	+	-	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	-	-
W002	Wheat	2011	Denholm, SK	C-PST-16	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	+	-	-	+	+	+	-	+
W018	Wheat	2011	Meaorth, AB	C-PST-17	+	+	-	+	-	+	+	-	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	-	-	-	+	+	+	-	-
W059	Wheat	2011	Lethbridge, AB	C-PST-18	+	+	-	-	-	+	+	-	+	-	-	+	+	-	-	-	+	+	+	-	-	-	-	+	-	-	-	+	+	+	-	-
W023	Wheat	2005	Winnipeg, MB	C-PST-19	+	+	-	+	-	+	+	-	+	-	-	+	+	-	-	+	+	+	+	+	-	-	-	+	-	+	-	+	+	+	-	-
W025	Wheat	2005	Winnipeg, MB	C-PST-20	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-
W058	Wheat	2007	Magrath, AB	C-PST-21	+	-	-	+	-	+	+	-	+	-	-	+	+	-	-	-	+	+	+	+	-	-	-	+	-	-	-	+	+	+	-	-
W010	Wheat	2011	Kenosee Lake, SK	C-PST-22	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	+	-	+	+	+	-	+	+	+	-	-
W040	Wheat	2013	Humboldt, SK	C-PST-23	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	-
W060	Wheat	2013	Lethbridge County, AB	C-PST-24	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	-	+
W036	Wheat	2013	Wadena, SK	C-PST-25	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-
W039	Wheat	2013	Insinger, SK	C-PST-26	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+
W056	Wheat	2011	Lacombe, AB	C-PST-27	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+
W016	Wheat	2011	Lethbridge, AB	C-PST-28	+	+	-	+	-	+	+	-	+	-	-	+	+	-	-	+	+	+	+	+	-	-	-	+	+	+	-	+	+	+	-	+
W008	Wheat	2011	Marriot, SK	C-PST-29	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	-	+	+	+	-	+	-	+	+	+	-	+
W015	Wheat	2010	Lethbridge, AB	C-PST-30	+	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	+	+	+	-	+
W049	Wheat	2013	Fairfield, AB	C-PST-31	+	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+
W031	Wheat	2011	Olds, AB	C-PST-32	+	+	-	+	-	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	-	-	+	-	+	+	+	-	+	+	+	-	+
W034	Wheat	2011	Bussano, AB	C-PST-33	+	+	-	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	-	+	+	+	-	-

APPENDIX-II. SNP details for all SNPs detected with >10X coverage in *Puccinia striiformis* f. sp. *tritici* genome.

SNP type	Base change	SNP count[a]	Percent[b]
Transitions	A>G	97,179	34.69
Transitions	C>T	96,602	34.48
Transversions	A>C	23,938	8.54
Transversions	A>T	21,366	7.63
Transversions	C>G	17,313	6.18
Transversions	G>T	23,755	8.48

[a]Number of each SNP (>10X coverage) type predicted; [b]Percent value overall

APPENDIX-III. Number of heterozygous SNPs (>10X coverage) for each *Puccinia striiformis* f. sp*. tritici* isolate.

Isolate No.	Isolate Name	No. of Het. SNPs	Percentage
1.	W088 (Old)	148,095	52.85
2.	W047 (Old)	145,756	52.01
3.	AR90-01(PST-3)	149,171	53.23
4.	AR97-01(PST-3)	145,295	51.85
5.	W021	126,819	45.25
6.	W023	126,659	45.20
7.	W024	129,805	46.32
8.	W025	129,893	46.35
9.	W058	144,139	51.44
10.	W055	129,933	46.37
11.	W015	158,370	56.51
12.	W017	128,298	45.78
13.	W018	144,586	51.59
14.	W054	156,344	55.79
15.	W028	140,434	50.11
16.	W031	129,986	46.38
17.	W034	127,187	45.39
18.	W056	80,258	28.64
19.	W001	127,766	45.59
20.	W002	127,216	45.40
21.	W003	127,083	45.35
22.	W004	129,643	46.26
23.	W005	126,723	45.22
24.	W006	127,090	45.35
25.	W007	126,252	45.05
26.	W008	129,424	46.18
27.	W009	123,422	44.04
28.	W010	126,789	45.24
29.	W011	140,713	50.21
30.	W012	126,581	45.17
31.	W030	129,321	46.15
32.	W052	164,630	58.75
33.	W053	139,578	49.81
34.	W013	129,439	46.19
35.	W019	129,170	46.09
36.	W029	153,042	54.61
37.	W037	129,283	46.13
38.	W038	129,518	46.22
39.	W039	131,611	46.96
40.	W040	129,347	46.16
41.	W042	129,158	46.09
42.	W043	129,856	46.34

43.	W044	129,473	46.20
44.	W045	140,416	50.11
45.	W046	129,716	46.29
46.	W049	131,394	46.89
47.	W051	132,994	47.46
48.	W060	152,791	54.52
Average	**--------**	**133,760**	**47.73**

APPENDIX -IV (Scientific report on stripe rust survey in SK in 2013, published in Canadian Plant Disease Survey, Volume 94, 124-126)

CROP / CULTURE: Winter Wheat, Spring Wheat, Barley
LOCATION / RÉGION: Saskatchewan

NAMES AND AGENCIES / NOMS ET ÉTABLISSEMENTS:
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TITLE / TITRE: STRIPE RUST OF WINTER WHEAT, SPRING WHEAT AND BARLEY IN SASKATCHEWAN IN 2013

ABSTRACT: Eighty-six commercial winter wheat crops, three wheat trap plots at Agriculture and Agri-Food Canada facilities, 17 commercial spring wheat and 30 commercial barley crops were surveyed for stripe rust in 2013 in Saskatchewan. Stripe rust was common on winter and spring wheat but uncommon on barley.

INTRODUCTION AND METHODS: Commercial crops of winter wheat (86), spring wheat (17) and barley (30), and susceptible wheat lines in the three trap plots, were surveyed at the late milk to soft dough stage of growth for stripe rust (*Puccinia striiformis* f. sp*. tritici* and *P. striiformis* f. sp*. hordei*) in 12 crop districts of Saskatchewan between early July and early September 2013. The crops surveyed were separated from each other by at least 20 km. Each crop was traversed in a 'V' pattern (Puchalski et al. 2012) within which individual plants, at five locations separated by about 40 m, were evaluated for incidence and severity of stripe rust. Incidence in each crop was estimated as the proportion of infected plants in a 5 m row per observation site exhibiting at least trace levels of stripe rust. The modified Cobb scale (Peterson et al. 1948) was used to estimate stripe rust severity on the flag leaves of 50 plants per crop (10 leaves per site). A six-category scale was used to summarize stripe rust severity in each field: clean (no visible symptoms); trace (<3% leaf area affected); light (3-5%); moderate (>15-20%); and severe (>20%).

RESULTS AND COMMENTS: Temperatures in Saskatchewan in 2013 were generally below normal for much of the growing season, but somewhat above normal beginning in late August and throughout September. There was limited precipitation in May, August and September, but precipitation was frequent in June and July. Rust teliospore formation and senescence of plant tissue were observed by mid-August.

Many commercial winter wheat crops in Saskatchewan were sprayed with foliar fungicides and thus it is likely that rust development was largely prevented. Stripe rust was observed in 26 winter wheat crops (30%), all three wheat trap plots, 8 spring wheat crops (47%), and 2 barley crops (7%). Of the 86 commercial winter wheat crops, 60 (70%) were rated as clean, 3 (3.5%) had trace levels, 11 (13%) were rated as light, 5 (6%) as moderate and 7 (8%) as having severe levels of stripe rust (Table 1). Stripe rust-susceptible winter and spring wheat genotypes in trap plots had moderate severity levels at Swift Current and severe ones at Melfort and Scott. The highest and lowest severity levels were found in Crop Districts 6B and 9A, respectively (Table 1). Severe infection was observed on an unsprayed crop of 'CDC Falcon' winter wheat at Insinger, SK in Crop District 5A. In spring wheat, stripe rust was most severe in crop district 8B and was observed at only trace levels in all other crop districts (Table 2). Only two barley crops, one in each of Crop Districts 6B and 8B, were affected by stripe rust. In both crops incidence was 3% and severity 5%.

REFERENCES:

Peterson, R.F., Campbell, A.B., and Hannah, A.E. 1948. A diagrammatic scale estimating rust intensity of leaves and stem of cereals. Can J. Res. Sect. C, 26:496-500.

Puchalski, B.J., Kundrik, K., Wogsberg, S., Wilson, M., Randhawa, H. and Gaudet D.A. 2012. Survey of stripe rust and other foliar diseases of wheat in 2012 in southern Alberta. Can. Plant Dis. Surv. 93:123-124. (www.phytopath.ca/cpds.shtml)

Table 1. Prevalence and severity categories for stripe rust on commercial winter wheat crops in 2013 in Saskatchewan by crop district.

Crop District	Prevalence*	Severity				
		Clean	Trace	Light	Moderate	Severe
2B	1/5	4	0	0	0	1
3A-N	2/4	2	0	2	0	0
3B-N	1/7	6	0	1	0	0
5A	4/9	5	1	0	2	1
5B	3/10	7	0	2	0	1
6A	3/11	8	0	2	1	0
6B	4/17	13	0	1	0	3
7A	1/4	3	0	1	0	0
7B	1/2	1	0	0	1	0
8A	0/5	5	0	0	0	0
8B	3/6	3	0	1	1	1
9A	3/6	3	2	1	0	0
Total	**29/89**	**60**	**3**	**11**	**5**	**7**

* proportion of crops or trap plots affected

Table 2. Prevalence and severity categories for stripe rust on commercial spring wheat crops in 2013 in Saskatchewan by crop district.

Crop District	Prevalence*	Severity				
		Clean	Trace	Light	Moderate	Severe
2B	0/4	4	0	0	0	0
3B-N	1/4	3	1	0	0	0
5A	1/1	0	1	0	0	0
5B	1/2	1	1	0	0	0
6A	2/3	1	2	0	0	0
8B	3/3	0	2	0	0	1
Total	**8/17**	**9**	**7**	**0**	**0**	**1**

* proportion of crops or trap plots affected

APPENDIX-V (Scientific report on stripe rust survey in SK in 2014, published in Canadian Plant Disease Survey, Volume 95, 110-111)

CROP / CULTURE: Winter and Spring wheat

LOCATION / RÉGION: Saskatchewan

NAMES AND AGENCIES/NOMS ET ETABLISSEMENTS:
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TITLE/TITRE: STRIPE RUST SURVEY OF WINTER AND SPRING WHEAT IN SASKATCHEWAN IN 2014

ABSTRACT: Thirty-eight winter and spring wheat crops and four plots with stripe rust differential wheat genotypes located at research establishments were surveyed for stripe rust in Saskatchewan in 2014. Stripe rust was found only in south-western Saskatchewan and was less wide-spread than in 2013.

INTRODUCTION AND METHODS: Thirty-eight commercial crops of winter and spring wheat and susceptible differential wheat lines in the four trap plots in 12 crop districts of Saskatchewan were surveyed at the late milk to soft dough stage for stripe rust (*Puccinia striiformis* f. sp*. tritici*) between late July and late August, 2014. The crops surveyed were separated from each other by at least 20 km. The Trap plots were located at Saskatoon (central SK), Prince Albert (northern SK), Scott (west-central SK) and Swift Current (southwestern SK). Each crop was traversed in a "V" pattern (Puchalski et al. 2013) within which individual plants from five sites separated by about 40 m were evaluated for incidence and severity of stripe rust. Incidence was estimated as the proportion of infected plants exhibiting at least trace levels of stripe rust in a 5 m row of the crop. The modified Cobb scale (Peterson et al. 1948) was used to assess stripe rust severity on the flag leaves of 50 plants per crop (10 leaves per site). A six-category scale was used to assess stripe rust severity in each field: clean (no visible symptoms); trace (<3% leaf area affected); light (3-5%); moderate (>15-20%); and severe (>20%).

RESULTS AND COMMENTS: In 2014, temperatures in Saskatchewan were slightly cooler than the long-term normal. In general, precipitation in all regions was close to the long term average in most months, except June, when precipitation was much greater than the long-term average.

Stripe rust was observed in 8 (21%) of the wheat crops in 2014 and on the susceptible differentials (i.e. Avocet -*YrA* and Avocet +*YrA*) in the four trap plots. Of the 38 wheat crops surveyed: 30 (79%) were clean and two (5%) had trace levels of stripe rust (Table 1). Additionally, 2 crops were rated as light, one as moderate and 3 as severe. Stripe rust was severe on Avocet -*YrA* (susceptible check) at Prince Albert and Scott and moderate at Saskatoon and Swift Current. Stripe rust was most widespread in crop district 4B with all four crops sampled being positive for stripe rust. The three severely affected crops were in crop districts 4A, 4B and 3B-N.

Stripe rust was frequently observed and uniformly distributed across the province in 2013 (Brar et al. 2014), but much less so in 2014. It was also light in 2014 in the USA as compared with 2013 (Anmin Wan, USDA Washington State, personal communication). This may be the reason for the low levels of stripe rust observed in Saskatchewan as the inoculum normally is blown in from the USA to the south. Stripe rust was observed in southern Alberta at moderate to severe levels in 2014 (André Laroche and Denis Gaudet, Agriculture and AgriFood Canada, Lethbridge, personal communication), which may be the reason for stripe rust mainly occurring in south-western Saskatchewan. In stripe rust-infected crops, teliospore formation was observed by late July to early August.

Stripe rust was not observed in 2014 in crop districts 5A, 9A-E, 9A-W, 8B, and 7A, which are in the northern and the eastern side of the province. This further supports the likelihood that stripe rust inoculum from southern Alberta spreads into south-western Saskatchewan.

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REFERENCES:

Brar, G., Liu, J., and Kutcher H.R. 2014. Stripe rust of winter wheat, spring wheat and barley in Saskatchewan in 2013. Can. Plant Dis. Survey. 94:124-126.

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Table 1. Prevalence (proportion of crops affected) and severity of stripe rust on commercial winter wheat crops in 2014 in Saskatchewan by crop district.

Crop District	Prevalence	Severity Class*				
		Clean	Trace	Light	Moderate	Severe
3A-N	0/4	4	0	0	0	0
3B-N	1/9	8	0	0	0	1
4A	1/2	1	0	0	0	1
4B	4/4	0	1	1	1	1
5A	0/2	2	0	0	0	0
6B	1/4	3	1	0	0	0
7A	0/2	2	0	0	0	0
7B	0/3	3	0	0	0	0
8B	0/1	1	0	0	0	0
9A-E	0/1	1	0	0	0	0
9A-W	0/2	2	0	0	0	0
9B	1/4	3	0	1	0	0
Total	**8/38**	**30**	**2**	**2**	**1**	**3**

*Severity classes: clean (no visible symptoms); trace (<3% leaf area affected); light (3-5%); moderate (>15-20%); severe (>20%)