MOLECULAR MAPPING OF A GENE FOR RESISTANCE TO STRIPE RUST IN SPRING WHEAT CULTIVAR IDO377S AND IDENTIFICATION OF A NEW RACE OF *Puccinia striiformis* f. sp. *tritici* VIRULENT ON IDO377S

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of PENG CHENG find it satisfactory and recommend that it be accepted.

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Chair

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Abstract

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important diseases of wheat worldwide. The best strategy to control stripe rust is growing resistant cultivars. To study the genetics of the stripe rust resistance in 'IDO377s' spring wheat, a cross was made between IDO377s and 'Avocet Susceptible' (AVS). Parents, F_2 plants, and F_3 lines of the cross were tested with races PST-43 and PST-45 of *P. striiformis* f. sp. *tritici* in the seedling stage under controlled greenhouse conditions. The results of genetic analyses showed a single dominant gene in IDO377s for resistance to these races. The resistance gene analog polymorphism (RGAP) and simple sequence repeat (SSR) techniques were used to identify molecular markers linked to the resistance gene. A total of 10 markers were identified, of which the two closest flanked the locus by 5.5 and 4.4 cM. The pattern of

presence and absence of two linked RGAP markers among the 21 nulli-tetrasomic lines of 'Chinese Spring' located the resistance gene to chromosome 2B. Testing ditelosomic line 2BL further refined the location of the gene to the long arm of chromosome 2B. The chromosomal location of the resistance gene was further confirmed by two 2BL- specific SSR markers and a sequence tagged site (STS) marker previously mapped to 2BL. Based on the chromosomal location and reactions to various races of the pathogen, the IDO377s gene, designated as *YrIDO377s*, is likely a novel gene different from all previously reported genes for resistance to stripe rust. A total of 108 wheat breeding lines and cultivars with IDO377s or related cultivars in their pedigree were used to validate the most closely linked flanking markers and select lines carrying *YrIDO377s*. The results showed that the flanking markers were reliable for assisting selection of breeding lines with the resistance gene. A new race, PST-127, was identified from an isolate collected from IDO377s in this study. How this new race would affect the use of *YrIDO377s* in breeding programs is discussed.

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DEDICATION

This thesis is dedicated to my parents, who always trust me and support me,

in love and gratitude.

CHAPTER ONE

LITERATURE REVIEW

1. Importance of Wheat

Wheat (*Triticum* spp.) is a cultivated grass worldwide and ranks second in acreage among the cereal crops after maize (*Zea mays* L.) and before rice (*Oryza sativa* L.) (USDA 2003). About one-fifth of the calories consumed by humans were provided by wheat with 620 million tons worldwide annual production (Statistic Division 2006). As a staple food used to make flour for breads, cookies, cakes, and noodles, the hexaploid common wheat (*T. aestivum*) is roughly 95% of the wheat crops. The remaining 5% is tetraploid durum wheat (*T. durum*), which is used for making pasta and other semolina products. Other cultivated species like einkorn (*T. monococcum*), emmer (*T. dicoccon*), and spelt (*T. spelta*) are no longer of economic importance.

The United States produces six classes of wheat: durum, hard red spring, hard red winter, soft red winter, hard white, and soft white. Club wheats are a special type of soft white wheat and tend to be lower in protein than soft white wheats. Hard wheat is often linked to high level of proteins, but is harder to process. Red wheat flour may need bleaching to make desirable food products.

Wheat originated in Southwest Asia in the area known as the Fertile Crescent. The domesticated einkorn (*T. monococcum*, genome A^mA^m) and emmer [*T. turgidum* subspecies (ssp.) *dicoccon*, genomes AABB] were expanded and disseminated from this area across Asia, Europe, and Africa (Dubcovsky and Dvorak 2007). The hexaploid common wheat (*T.*

aestivum, genomes AABBDD) is a hybridization of either domesticated emmer or durum wheat with another wild diploid grass *Aegilops tauschii* (genome DD) (Kihara 1944; McFadden and Sears 1946). Dvorak et al. (1998) suggested that the principal area of the origin of *T. aestivum* is within the corridor stretching from Armenia to the southwestern coastal area of the Caspian Sea.

The important traits for wheat domestication were loss of spike shattering and tough glumes, increased seed size, reduced number of tillers, more erect growth, and reduced seed dormancy (Dubcovsky and Dvorak 2007). Wheat populations of traditional agricultural systems often consist of landraces. These informal farmer-maintained populations usually maintain high levels of genetic diversity. The superior seeds with beneficial traits were selected by hand and planted for next year harvest. This forms the ancient type of plant breeding. Modern wheat breeding was triggered by the rediscovery of the Mendel's laws in the first years of the twentieth century. The standard method of breeding inbred wheat cultivars is hybridization of two lines by using hand emasculation and pollination. The progeny of the cross are selected several generations for the special traits before release as a variety or cultivar. The major traits that breeders are interested in include yield components, physiological traits, canopy-based traits, stressed environments, and disease resistance (Hayward et al., 1993).

During the past two decades, molecular tools have aided tremendously in the identification, mapping, and isolation of genes in a wide range of crop species. Carrying out indirect selection through molecular markers linked to the traits of interested, both single genes and quantitative trait loci (QTL), improved the efficiency of conventional plant

breeding. Some traits, especially those have nothing to do with the environment, can be selected through marker-assisted selection (Gupta et al. 1999).

Wheat has also been used as a model system for the study of polyploid cytogenetics because of the ease of chromosome manipulation. The haploid bread wheat genome size is about 1.7×10^{10} bp with an average of 810 Mb per chromosome (Arumuganathan and Earle 1991). The average wheat chromosome is 25-fold longer than that of rice (Moore et al. 1995). Such a large genome of bread wheat resulted from polyploid, as well as extensive duplication, which has clustered genes in small chromosome regions that have high frequencies of genetic recombination (Gill and Gill 1994). These small chromosome regions are more suitable for molecular manipulations compared to other crops with small genomes.

2. Wheat Stripe Rust

Wheat stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is a destructive disease and has been reported in over 60 countries (Chen 2005). Transcaucasia, the origin of domesticated wheat, also is assumed as the center of origin for *P. striiformis*. The pathogen might have moved from the primary host grasses into Europe and along the mountain ranges to China and eastern Asia (Stubbs 1985). Although the pathogen was first recognized in North America in 1915 by a visiting scientist, F. Kolpin Ravn, from Denmark, it is believed that *P. striiformis* had existed in North America for at least 23 years before this first report (Line 2002). According to Smith's disease report in 1961 (Smith, 1961), the earliest report of wheat rust could be traced back to the 1700s in California and the rust was likely stripe rust.

Schmidt first described the stripe rust pathogen on barley glumes in Europe as *Uredo glumarum* in 1827 (Humphrey et al., 1923). Eriksson and Henning in 1894 named the fungus *Puccinia glumarum* based on its telial stage (Humphrey and Johnson, 1916). The currently accepted name *Puccinia striiformis* was proposed by Cummis and Stevenson in 1956 (Cummis and Stevenson 1956).

The fungal pathogen stripe rust is in the order of Puccineales of Basidiomycetes. Like many other fungal pathogens, moist conditions are needed for the pathogen to infect host plants. The disease more likely occurs in relatively low temperatures compared to leaf rust (*P. triticinia* Eriks.) and stem rust (*P. graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.). About one week after infection, symptoms can be observed and sporulation starts in about two weeks under favorable conditions. Yellow to orange color rust pustules (uredia) formed, which contain thousands of urediniospores. Unlike leaf rust and stem rust, stripe rust does not have any known alternate hosts for basidiospores to infect. Thus, there are only dikaryotic uredial, dikaryotic and diploid telial, and haploid basidial stages in its life cycle, without pycnial and aecial stages (Chen 2005).

The germination of urediniospores has an optimal temperature of 7-12°C. Newton and Johnson (1936) reported that *P. striiformis* spores could germinate under a minimum temperature above 0°C. High temperature inhibits the growth of the fungus or induces the pathogen to enter dormancy. They also reported that plants were susceptible at 13°C and resistant at 25°C. Unlike other rusts of wheat, the binucleate urediniospores of *P. striiformis* enter the plant cell through germ tubes without forming obvious appressoria (Marryat 1907). There are two nuclei in the vesicle, four nuclei in the infection hyphae which later were

multinuclear. Hungerford demonstrated that stripe rust could overwinter by mycelia dormancy and urediniospores (Hungerford 1923).

Puccinia striiformis has a wide host range including common wheat, durum, spelt, and emmer wheat, barley, and rye. Eriksson (1894) named five formae speciales of the pathogen based on the host genus (*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.). Collections from orchard grass (*Dactylis glomerata* L.) were designated as *P. striiformis* f. sp. *dactylidis* (Manners 1960; Tollenaar 1967); collections from Kentucky blue grass (*Poa pratensis* L.) were designated as *P. striiformis* f. sp. *poae* (Britton and Cummins 1956; Tollenaar 1967), and collections from *Leymus secalinus* (Georgi) Tzvel were designated as *P. striiformis* f. sp. *leymi* (Niu et al. 1991). Even the subdivision of *P. striiformis* into formae speciales has been questioned because of the presence of overlapped hosts in two different formae speciales, this subdivision was supported by the greenhouse and field data (Stubbs 1985; Zadoks 1961). The virulence and random-amplified polymorphic DNA (RAPD) analyses conducted by Chen et al. (1995) clarified the relationships among *P. striiformis* f. sp. *hordei*, *P. striiformis* f. sp. *tritici*, and *P. striiformis* f. sp. *poae*. *Puccinia striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* were more closely related to each other than they were to *P. striiformis* f. sp. *poae*. Besides formae speciales, race has been used to distinguish the isolates of *P. striiformis* based on their virulence/avirulence patterns on differential cultivars of wheat or barley. The virulence/ avirulence patterns of an isolate define the race designation. In the United States, 20 wheat genotypes (Table 1) are currently used to differentiate races of *P. striiformis* f. sp.

tritici (Chen et al. 2002).

TABLE 1. Wheat genotypes used to differentiate races of *Puccinia striiformis* f. sp. *tritici* in the United States.

Differential No.	Cultivar or line	*Yr* gene
1	Lemhi	*Yr21*
2	Chinese 166	*Yr1*
3	Heines VII	*Yr2, YrHVII*
4	Moro	*Yr10, YrMor*
5	Paha	*YrPa1, YrPa2, YrPa3,*
6	Druchamp	*Yr3a, YrD, YrDru*
7	Yr5	*Yr5*
8	Produra	*YrPr1, YrPr2*
9	Yamhill	*Yr2, Yr4a, YrYam*
10	Stephens	*Yr3a, YrS, YrSte*
11	Lee	*Yr7, Yr22, Yr23*
12	Fielder	*Yr6, Yr20*
13	Tyee	*YrTye*
14	Tres	*YrTr1, YrTr2*
15	Hyak	*Yr17, YrTye*
16	Express	*YrExp1, YrExp2*
17	Yr8	*Yr8*
18	Yr9	*Yr9*
19	Clement	*Yr9, YrCle*
20	Compair	*Yr8, Yr9*

For rust fungi, virulence/avirulence patterns are characterized based on infection type (IT)

scores. The degree of visible pustules on the plant surface and the degree of necrosis and chlorosis of the plant tissue determine IT. Line and Qayoum (1992) described a 0-9 scale for recording *P. striiformis* IT as follows: 0, no visible signs or symptoms; 1, necrosis and/or chlorotic flecks, no sporulation; 2, necrosis and/or chlorotic blotches or stripes, no sporulation; 3, necrosis and/ or chlorotic blotches or stripes, trace sporulation; 4, necrosis and/or chlorotic blotches or stripes, light sporulation; 5, necrosis and/or chlorotic blotches or stripes, intermediate sporulation; 6, necrosis and/or chlorotic blotches or stripes, moderate sporulation; 7, necrosis and/or chlorotic blotches or stripes, abundant sporulation; 8, chlorosis behind sporulating area, abundant sporulation; and 9, no necrosis or chlorosis, abundant sporulation. Usually, ITs 0-3 were considered as an avirulent phenotype for the pathogen (resistant for the plant; incompatible for the interaction), ITs 4-6 were considered as an intermediate phenotype, and ITs 7-9 were considered as a virulent phenotype (susceptible; compatible). Since the pathogen evolves rapidly, addition of new differentials with new resistance genes is desired to identify new races.

3. Control of wheat stripe rust

Although fungicides are effective for control of stripe rust since their first large-scale use in North America in 1981 (reviewed by Line 2002), the application of fungicides adds substantial cost to wheat production. The use of fungicides presents both economic and health problems to many growers, especially in developing countries. Problems raised by the chemical control also include adverse affects on the environment and elicitation of fungicide-resistant strains of the pathogen. The best strategy to control stripe rust is to grow

resistant cultivars. Types of resistance to stripe rust can be generally separated into two categories: all-stage resistance (also called seedling resistance) and adult-plant resistance such as the high temperature, adult-plant (HTAP) resistance (Table 2). All-stage resistance is typically race-specific and can be detected at the seedling stage but is also expressed at all growth stages of plant (reviewed by Chen 2005). Rapid development of new virulent races of the pathogen due to mutation and somatic recombination makes cultivars with all-stage resistance become susceptible very soon after they have been released (Wellings and McIntosh 1990; Line and Qayoum 1992; Chen 2005, 2007). This is because all-stage resistance is often conferred by single genes. In contrast, HTAP resistance is non-race specific, durable, and often quantitatively inherited (Qayoum and Line 1985; Chen and Line 1995a, 1995b; Line 2002; Chen 2005). Plant growth stage, temperature, and humidity are three major factors that affect HTAP resistance.

TABLE 2. Comparison of all-stage resistance and high-temperature and adult-plant (HTAP) resistance

	All-stage resistance	HTAP resistance
Number of gene conferred	Single genes	QTL
Durability	Not durable	Durable
Effect stage	All stages	Adult plant
Specificity	Race-specific	Non race-specific
Level of resistance	High	Partial
Mode of inheritance	Qualitatively	Quantitatively

To test and differentiate all-stage and HTAP resistance in cultivars, the routine method used in Chen's program is: cultivars are first tested with as many races as possible under the standard low diurnal temperature cycle (gradually changing from 4°C at 2 am to 20°C at 2 pm) to identify races virulent on the seedlings, and then, adult-plants (boot to heading stage) of the cultivars are tested at the standard high diurnal temperature cycle (gradually changing from 10°C at 2 am to 35°C at 2 pm) with selected races that are virulent to the cultivars in the seedling tests. The cultivars with HTAP resistance should have lower ITs in the adult-plant stages than those in seedling tests. In the Pacific Northwest, 90% of the released wheat cultivars have HTAP resistance because of its non-race specific nature, durability, and effectiveness (Line, 2002).

4. Resistance to stripe rust

Biffen (1905) first demonstrated that resistance to stripe rust follows Mendel's laws. Most of the resistance genes were identified after 1960. Currently, more than 40 stripe rust resistance genes with official *Yr* designations have been reported in wheat. More than 100 genes and QTLs have been temporarily named (USDA cereal disease lab, Catalog of Rust Resistance Genes in Small Grains. http://www.ars.usda.gov/Main/docs.htm?docid=10342). Standard methods widely used to study the number of resistance genes and the nature of resistance including making crosses between resistant and susceptible cultivars and testing the F_2 or later generation with specific stripe rust races.

As reviewed in Table 3, 40 officially designated *Yr* genes, and 51 temporarily designated *Yr* genes have been reported. Most of the 14 genes reported after Chen's review (2005) are

novel based on the resources of wheat genotypes or wild species, their chromosomal locations, and responses to pathotypes.

QYrtm.pau-2A and *QYrtb.pau-5A* were mapped using a set of 93 RILs generated from a cross involving *T. monococcum* (acc. pau14087) and *T. boeoticum* (acc. pau5088) (Chhuneja et al. 2008). These two QTLs were believed to confer HTAP resistance based on the parental accessions test with a mixture of pathotypes in the field condition for three years. All the tested RILs were susceptible at the seedling stage but resistant at the adult-plant stage. The *Lr56/Yr38* loci which were temporarily designated as *YrS12/LrS12* (Marais et al. 2003) were reported to confer resistance to both leaf rust and stripe rust. Introgressed from *Aegilops sharonensis*, the resistance genes occurred on chromosome 6A and showed non-Mendelian transmission (Marais et al. 2006). *Yr39* and *YrAlp* identified from spring wheat cultivar Alpowa conferred HTAP and all-stage resistance to stripe rust, respectively (Lin and Chen 2007). *Yr40* was transferred from *Ae. geniculata* and characterized from a cryptic alien introgression line by inducing homologous chromosome pairing between wheat chromosome 5D and 5Mg of *Ae. geniculata* (Kuraparthy et al. 2007).

As summarized in Table 3, wild species have been important sources of rust resistance genes. The successful transfer and characterization of alien introgression is needed to introduce and identify new resistance genes in wheat. *Aegilops* species have provided resistance genes against multiple diseases, especially stripe rust, leaf rust, and stem rust (Marais et al. 2006, Kuraparthy et al. 2007, Mago et al. 2005).

All stage resistance conferred by single dominant genes provides high level of resistance, but are typically circumvented by new races. In contrast, HTAP resistance controlled by QTL

provides durable resistance, but is partial and harder to incorporate into new cultivars. Therefore, a combination of the two types of resistance genes in wheat cultivars should provide high level and durable resistance. More genes are needed to increase the diversity of resistance used in breeding programs and for monitoring virulence changes in the stripe rust population. In the current study, I identified a gene in wheat cultivar IDO377s that provides resistance to the predominant races of *P. striiformis* f. sp. *tritici* and identified flanking markers useful for marker-assisted selection. Although a new race identified in 2007 is virulent on IDO377s, the resistance gene still can be useful in combination with other resistance genes. The resistance gene in IDO377s should help us to understand the recent virulence changes in the stripe rust population in the United States.

Table 3. Genes and quantitative trait loci (QTL) for resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*), examples of wheat genotypes containing the genes, their chromosomal locations, types of resistance, and references

Yr gene	Example of wheat genotype	Chromosomal location	Resistance type[a]	Reference
Yr1	Chinese 166	2AL	RS, AS	Lupton and Macer 1962
Yr2	Heines VII	7B	RS, AS	Lupton and Macer 1962
Yr3a	Cappelle Desprez	1B	RS, AS	Lupton and Macer 1962
Yr3b	Hybrid 46		RS, AS	Lupton and Macer 1962
Yr3c	Minister	1B	RS, AS	Lupton and Macer 1962
Yr4a	Cappelle Desprez	6B	RS, AS	Lupton and Macer 1962
Yr4b	Hybrid 46	6B	RS, AS	Lupton and Macer 1962
Yr5	*Triticum aestivum* subsp. *spelta* Album	2BL	RS, AS	Macer 1966
Yr6	Heines Kolben	7BS	RS, AS	Macer 1966
Yr7	Lee	2BL	RS, AS	Macer 1966
Yr8c	Compair	2D (2A, 3D)	RS, AS	Riley et al. 1968
Yr9	Clement	1RS/1BL	RS, AS	Macer 1975
Yr10	Moro	1BS	RS, AS	Macer 1975

Yr11	Joss Cambier		RS, AP	McIntosh 1988
Yr12	Frontier		RS, AP	McIntosh 1988
Yr13	Hustler		RS, AP	McIntosh 1988
Yr14	Kador		RS, AP	McIntosh 1988
Yr15	*Triticum turgidum* var. *dicoccoides* G-25	1BS	RS, AS	Gerechter-Amitai et al. 1989
Yr16	Bersee	2D	NRS, AP	Worland and Law 1986
Yr17	VPM1	2AS	RS, AS	Bariana and McIntosh 1993
Yr18	Jupateco 73R	7DS	NRS, HTAP	Singh 1992
Yr19	Compair	5B	RS, AS	Chen et al. 1995b
Yr20	Fielder	6D	RS, AS	Chen et al. 1995b
Yr21	Lemhi	1B	RS, AS	Chen et al. 1995b
Yr22	Lee	4D	RS, AS	Chen et al. 1995b
Yr23	Lee	6D	RS, AS	Chen et al. 1995b
Yr24	Yr24/6*AVS	1BS	RS, AS	McIntosh et al. 1998
Yr25	Strubes Dickkopf	1D	RS, AS	McIntosh et al. 1998
Yr26	R55	1BS	RS, AS	McIntosh et al. 1998

Yr27	Ciano 79	2BS	RS, AS	McDonald et al. 2004
Yr28	Synthetic	4DS	RS, AS	Singh et al. 2000
Yr29	Pavon F76	1BL	NRS, AP	McIntosh et al. 2001
Yr30	Opata 85	3BS	NRS, AP	McIntosh et al. 2001
Yr31	Pastor	2BS	RS, AS	McIntosh et al. 2003
Yr32	Carstens V	2AS	RS, AS	Eriksen et al. 2004
Yr33	Batavia	7DL	RS, AS	McIntosh et al. 2004
Yr34	WAWHT2046	5AL	AP	McIntosh et al. 2004
Yr35	98M71	6BS	RS, AS	Marais et al. 2005
Yr36	Glupro', RSL No.65	6BS	NRS, HTAP	Uauy et al. 2005
Yr37	S14	2DL	RS, AS	Marais et al. 2005
Yr38	*Aegilops sharonenisis*	6A	RS, AS	Marais et al. 2006
Yr39	Alpowa	7BL	NRS, HTAP	Lin and Chen 2007
Yr40	T5DLC.5DS-5MgSa	5DS	RS, AS	Kuraparthy et al. 2007
YrH52	*T. turgidum* var. *dicoccoides* H52	1BS	RS, AS	Peng et al. 2000
Yrns-B1	Lgst. 79-74	3BS	NRS, AP	Börner et al. 2000

YrSP	Spaldings Prolific	2BS	RS, AS	McIntosh et al. 1995
YrA	Anza		RS, AS	McIntosh et al. 1998
YrCle	Clement	4B	RS, AS	Chen et al. 1998a
YrDru	Druchamp	5B	RS, AS	Chen et al. 1998a
YrDru2	Druchamp	6A	RS, AS	Chen et al. 1998a
YrDa1	Daws	1A	RS, AS	Chen et al. 1998a
YrDa2	Daws	5D	RS, AS	Chen et al. 1998a
YrH46	Hybrid 46	6A	RS, AS	Chen et al. 1998a
YrHVII	Heines VII	4A	RS, AS	Chen et al. 1998a
YrMin	Minister	4A	RS, AS	Chen et al. 1998a
YrMor	Moro	4B	RS, AS	Chen et al. 1998a
YrND	Nord Desprez	4A	RS, AS	Chen et al. 1998a
YrSte	Stephens	2B	RS, AS	Chen et al. 1998a
YrSte2	Stephens	3B	RS, AS	Chen et al. 1998a
YrTye	Tyee	6D	RS, AS	Chen et al. 1998a
YrTr1	Tres	6D	RS, AS	Chen et al. 1998a

YrTr2	Tres	3A	RS, AS	Chen et al. 1998a
YrYam	Yamhill	4B	RS, AS	Chen et al. 1998a
YrV23	Vilmorin 23	2B	RS, AS	Chen et al. 1998a
YrJh1	Jinghe 8811	2A	RS, AS	Zhang et al. 2001
YrJh2	Jinghe 8811	4D	RS, AS	Zhang et al. 2001
YrGui1	Guinong 22		RS, AS	Cao et al. 2004
YrGui2	Guinong 22		RS, AS	Cao et al. 2004
YrGui3	Guinong 22		RS, AS	Cao et al. 2004
YrJu1	Jubilejna II		RS, AS	Zhao et al. 2004
YrJu2	Jubilejna II		RS, AS	Zhao et al. 2004
YrJu3	Jubilejna II		RS, AS	Zhao et al. 2004
YrJu4	Jubilejna II		RS, AS	Zhao et al. 2004
YrA1	Gaines, Nugaines		NRS, HTAP	Chen et al. 1998a
YrA2	Nugaines		NRS, HTAP	Chen et al. 1998a
YrA3	Luke		NRS, HTAP	Chen et al. 1998a
YrA4	Luke		NRS, HTAP	Chen et al. 1998a

YrA5	Druchamp		NRS, HTAP	Chen et al. 1998a
YrA6	Druchamp		NRS, HTAP	Chen et al. 1998a
YrA7	Stephens	6BS	NRS, HTAP	Chen et al. 1998a
YrA8	Stephens		NRS, HTAP	Chen et al. 1998a
YrAlp	Alpowa	1BL	RS, AS	Lin and Chen 2007
YrC108	CI108		RS, AS	He et al. 2007
YrCH42	Chuanmai 42	1B centromere	RS, AS	Liu et al. 2006
YrChk	Chike	1BL	RS, AS	Liu et al. 2007
YrCN19	AIM6	2BS	RS, AS	Luo et al. 2005
YrHua	H9020-17-15	6AL	RS, AS	Cao et al. 2008
YrTp1	*Thinopyrum ponticum*	2BS	RS, AS	Yin et al. 2006
YrTp2	*Thi. ponticum*	7BS	RS, AS	Yin et al. 2006
Yr-XU	PI31 from Syria common wheat line Xu18	1BS	RS, AS	Pu et al. 2006
YrExp1	Express	1BL	RS,AS	Lin and Chen 2008
YrExp2	Express	5BL	RS,AS	Lin and Chen 2008
YrCK	Cook	2DS	APR	Navabi et al 2005

YrZH84	Zhou 8425B	7BL	RS, AS	Li et al. 2006
QPst.jic-1B	Guardian	1BL	APR	Melichar et al. 2008
QPst.jic-2D	Guardian	2D	APR	Melichar et al. 2008
QPst.jic-4B	Guardian	4B	APR	Melichar et al. 2008
QYr.inra-2AL	Re´cital/Camp Re´my (CR)	2AL	AP	Mallard et al. 2005
QYr.inra-2BL	Re´cital/Camp Re´my (CR)	2B centromere	AS	Mallard et al. 2005
QYr.inra-2BS	Re´cital/Camp Re´my (CR)	2BS	AP	Mallard et al. 2005
QYr.inra-2DS	Re´cital/Camp Re´my (CR)	2DS	AP	Mallard et al. 2005
QYr.inra-5BL.1	Re´cital/Camp Re´my (CR)	5BL	AP	Mallard et al. 2005
QYr.inra-5BL.2	Re´cital/Camp Re´my (CR)	5BL	AP	Mallard et al. 2005
QYrst.wgp-6BS.1	Stephens	6BS	NRS, HTAP	Santra et al. 2008
QYrst.wgp-6BS.2	Stephens	6BS	NRS, HTAP	Santra et al. 2008
QYrtb.pau-5A	*T. boeoticum*	5A	NRS, HTAP	Chhuneja et al. 2008
QYrtm.pau-2A	*T. monococcum*	2A	NRS, HTAP	Chhuneja et al. 2008

[a] RS = race specific resistance; AS = all-stage resistance; AP = adult plant resistance; HTAP = high-temperature adult plant resistance; NRS = non-race specific resistance.

5. Molecular markers for resistance genes

To map resistance genes, bulk segregant analysis (BSA) (Michelmore et al. 1991) has been widely used. For BSA, a number of resistant progeny selected from the disease test are used as a resistant bulk and a number of susceptible progeny selected from the disease test are used as a susceptible bulk. Markers were screened with genomic DNA of the resistant parent, resistant bulk, susceptible parent, and susceptible bulk to look for markers that resistance associated or susceptibility associated.

Several techniques have been used to develop molecular markers for resistance to stripe rust. Widely used marker techniques include random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), and amplified fragment length polymorphism (AFLP). The RAPD technique employs 10 base pair random primers to amplify random segments of genomic DNA to reveal polymorphisms. The DNA is hybridized by the primers at specific sites and amplified into many specific-length segments that can be separated by gel electrophoresis (Williams et al. 1990). The SSR technique employs primers designed based on short sequences of nucleotides (2-6 units in length) that are repeated in tandem among the genome (Jacob et al. 1991). AFLP uses restriction enzymes to cut genomic DNA, followed by ligation of complementary double stranded adaptors to the ends of the restriction fragments (Vos et al. 1995). A subset of the restriction fragments are then amplified using two primers complementary to the adaptor and restriction site sequences. The resulting fragments are visualized on denaturing polyacrylamide gels either through autoradiography or fluorescence methodologies.

The resistance gene analog polymorphism (RGAP) technique is a molecular marker technique using high resolution electrophoresis and sensitive detection of DNA fragments amplified with primers based on conserved domains of plant resistance genes (Chen et al. 1998). Because it is efficient, reliable, low-cost, and high possibility to developing direct

markers for disease resistance, the RGAP technique has been used extensively, to identify genetic variation of *Pinus oocarpa* populations (Díaz and Ferrer 2003), study the evolution of resistance gene in peanut (Yuksel et al. 2005), mapping *Rpi-ber* gene resistant to potato late blight (Hori et al. 2006), and QTL analysis of *Fusarium* head blight in barley (Rauscher et al. 2006). Chen and his colleagues used the technique to identify markers for numerous genes or QTL in wheat and barley for resistance to stripe rust and other diseases (Shi et al. 2001; Chen and Yan 2002; Yan et al. 2003; Pahalawatta and Chen 2005a, 2005b; Yan and Chen 2006, 2008; Lin and Chen 2007, 2008).

As shown in Table 4, molecular markers have been developed for numerous stripe rust resistance genes. These markers helped identify the genes and were used to map them to wheat chromosomal locations. However, not all of these markers are useful in marker-assisted selection, especially those not tightly linked to the target genes. In general, more closely linked markers need to be found. To reduce the possibility of selecting wrong breeding materials using linked markers, flanking markers are commonly used. This point has been demonstrated by Chen and his associates (Yan and Chen 2007, 2008; Lin and Chen 2008).

A good marker should be highly polymorphic in addition to being tightly linked to a resistance gene. In some cases, even a co-segregating marker cannot be used because of the lack of polymorphism between marker alleles of genotypes with the gene and genotypes without the gene (Chen et al. 2003). The cleaved amplified polymorphic sequence (CAPS) marker technique may sometimes solve the problem (Chen et al. 2003). To determine how useful a molecular marker is in indicating the resistance gene, validation of the markers with plant genotypes with and without the gene is needed. Also, the markers should be tested with a number of plant genotypes to determine the polymorphism at the marker loci (Yan and Chen 2007, 2008; Lin and Chen 2008).

TABLE 4. Molecular markers for genes and quantitative trait loci (QTL) for resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*), primer name and sequence for the markers, marker size, distance, and references.

Yr gene or QTL	Markers	Primer names	Primer sequence	Marker size(bp)	Distance (cM)	References
Yr5	*S19M93-140*	S19M93-100F	TAATTGGGACCGAGAGACG	100	0	Smith et al. 2007
		S19M93-100R	TTCTTGCAGCTCCAAAACCT			
	Xwgp-17	S2	GGIGGIGTIGGIAAIACIAC	546	0	Yan et al. 2003
		AS3	IAGIGCIAGIGGIAGICC			
	Yr5STS-7/8	Yr5STS-7	GTACAATTCACCTAGAGT	478, 472	0	Chen et al. 2003
		Yr5STS-8	GCAAGTTTTCTCCCTATT			
Yr9	*IB-267*		GCAAGTAAGCAGCTTGATTTAGC	267	0	Mago et al. 2002
			AATGGATGTCCCGGTGAGTGG			
	Xwgp8	Xa1NBS-R	CTCTGTATACGAGTTGTC	365	0	Shi et al. 2001
		XLRR-INV2	GAGGAAGGACAGGTTGCC			
Yr10	*Xpsp3000*		GCAGACCTGTGTCATTGGTC	286	1.2	Wang et al. 2002
			GATATAGTGGCAGCAGGATACG			
Yr15	*Nor1*			2800	2.6	Chagué et al., 1999, Peng et al. 2000
	UBC212a (Xgwm413)		TGCTTGTCTAGATTGCTTGGG	1100	4.3	
			GATCGTCTCGTCCTTGGCA			
Yr17	*(SC-Y15)*			580	0.8 ± 0.7	Robert et al.,

						1999
Yr18	*Xgwm1220*				0.9	Spielmeyer et al. 2005
	Xgwm295		GTGAAGCAGACCCACAACAC	254	2.7	
			GACGGCTGCGACGTAGAG	258		
Yr26 (YrCH42, Yr24)	*CHR-4*	Pto-kin2IN	GATGCACCACCAGGGGG	572	1.5	Wen, te al 2008
		Xa1LR-R	GAGATTGCCAAGCAATTGC			
	CHR5	Pto-kin1	GCATTGGAACAAGGTGAA	355	0.5	
		S2	GGIGGIGTIGGIAAIACIAC			
Yr27	*Xcdo405-2B*	M13forward	GTAAAACGACGGCCACT			McDonald et al 2004
		M13reverse	AACAGCTATGACCATG			
	Xbcd152-2B	M13forward	GTAAAACGACGGCCACT			
		M13reverse	AACAGCTATGACCATG			
Yr28	*Xmwg634-4DS*					Singh et al. 2000
Yr29	*Xwmc44*		GGTCTTCTGGGCTTTGATCCTG	242	3.6	Rosewarne et al 2006
			TGTTGCTAGGGACCCGTAGTGG			
	XTG818				2.1	
	XBac17R				2.1	
Yr32	*M62/P19–156*				0	Eriksen et al 2004
	Xwmc198-2AL		CACGCTGCCATCACTTTTAC	159	2	
			TTGAAGTGGTCATTGTTGCT			

Yr33	*a 7DL marker*					McIntosh et al. 2004
Yr34	*B1*				12.2	Bariana et al. 2006
Yr36	*Xbarc101*		GCTCCTCTCACGATCACGCAAAG		2	Uauy et al 2005
			GCGAGTCGATCACACTATGAGCCAATG			
Yr39	*Xwgp36*	Pto kin1	GCATTGGAACAAGGTGAA	830	0.8	Lin and Chen 2007
		RLK For	GAYGTNAARCCIGARAA			
	Xwgp45	Pto kin1	GCATTGGAACAAGGTGAA	940	6.6	
		XLRR For	CCGTTGGACAGGAAGGAG			
Yr40	*Gsp*					Kuraparthy et al. 2007
	xfbb276		AACAGCTATGACCATG			
			GTAAAACGACGGCCAGT			
YrA7	*Xgwm88.2*					Santra et al. 2005
	Xucw71		CTTGCACCCGTGGATCAG			
			CGATGCAATAATTTATCACACGTA			
YrAlp	*Xwgp47*	RLK For	GAYGTNAARCCIGARAA	1120	1.1	Lin and Chen 2007
		Pto kin2IN	GATGCACCACCAGGGGG			
	Xwgp48	Pto kin1	GCATTGGAACAAGGTGAA	450	3.2	
		Pto kin3	TAGTTCGGACGTTTACAT			
YrCH42	*Xgwm498*		GGTGGTATGGACTATGGACACT	160	1.6	Liu et al. 2006

			TTTGCATGGAGGCACATACT			
	Xbarc187		GTGGTATTTCAGGTGGAGTTGTTTTA		2.3	
			CGGAGGAGCAGTAAGGAAGG			
YrChk	*Xwmc44*		GGTCTTCTGGGCTTTGATCCTG	242	8.3	Liu et al. 2007
			TGTTGCTAGGGACCCGTAGTGG			
	Xgwm259		AGGGAAAAGACATCTTTTTTTTC	105	9.1	
			CGACCGACTTCGGGTTC			
YrCN19	*Xgwm410*		GCTTGAGACCGGCACAGT			Luo et al. 2005
			CGAGACCTTGAGGGTCTAGA			
YrExp1	*Xwgp78*	XLRR For	CCGTTGGACAGGAAGGAG	470	4.2	Lin and Chen 2008
		XLRR-INV1	TTGTCAGGCCAGATACCC			
	Xwmc631	L	TTGCTCGCCCACCTTCTACC	220/180	3.4	
		R	GGAAACCATGCGCTTCACAC			
YrExp2	*Xwgp81*	XLRR Rev	CCCATAGACCGGACTGTT	855	1	Lin and Chen 2008
		Pto kin3	TAGTTCGGACGTTTACAT			
	Xwgp82	XLRR Rev	CCCATAGACCGGACTGTT	895/880	0.7	
		Pto kin2	AGGGGGACCACCACGTAG			
YrH52	*Nor1*				1.4	Peng et al 1999
YrHua	*PM14(STS)*	F	GTACATGCAGACAGAAAGAGAGAA	296	5.4	Cao et al. 2008
		R	TGATGAGTCCTGAGTAACTC			

	PM42				2.7	
YrMor	*S26M47-150*	S26M47Forward	TTTACAGGTTGGAATCTA	150	0	Smith et al. 2002
		S26M47Reverse	GAATATACCTTTTCTTCAA			
Yrns-B1	*Xgwm1329*			152	<2.5	Khlestkina et al. 2007
YrSpP	*WMC441*		TCCAGTAGAGCACCTTTCATT	158	12.1	Guan et al. 2005
			ATCACGAAGATAAACAAACGG			
YrZH84	*Xcfa2040-7B*		TCAAATGATTTCAGGTAACCACTA	286	1.4	Li et al 2006
			TTCCTGATCCCACCAAACAT			
	Xbarc32-7B		GCGTGAATCCGGAAACCCAATCTGTG		4.8	
			TGGAGAACCTTCGCATTGTGTCATTA			
QPst.jic-1B	*Xgwm818*					Melichar et al 2008
	Xgwm259		AGGGAAAAGACATCTTTTTTTTC			
			CGACCGACTTCGGGTTC			
QPst.jic-2D	*Xgwm539*		CTGCTCTAAGATTCATGCAACC			Melichar et al 2008
			GAGGCTTGTGCCCTCTGTAG			
	Xgwm349		GGCTTCCAGAAAACAACAGG			
			ATCGGTGCGTACCATCCTAC			
QPst.jic-4B	*Xwmc652*		ATACGGCAAAGGAGAAGCGG	164		Melichar et al 2008
			GGTAGCGCTAATGCAGGGTG			
	Xwmc692		TTATCTTGATCCGAGCGA	131		

			ATGTGATTAGTCCTAAGGTCTCTCT			
QYr.inra-2AL	*Xgwm382a*		GTCAGATAACGCCGTCCAAT		13.98	Mallard et al 2005
			CTACGTGCACCACCATTTTG			
	Xgwm359		CTAATTGCAACAGGTCATGGG		5.52	
			TACTTGTGTTCTGGGACAATGG			
QYr.inra-2BS	*Xgpw3032*				18.1	Mallard et al 2005
	Xcfd50a		TTCTGCAACATTTTGTCCCA	261	12	
			CGTATGATCCTAACGAGGGC			
QYr.inra-2BL	*Xbarc101*		GCTCCTCTCACGATCACGCAAAG		1.2	Mallard et al 2005
			GCGAGTCGATCACACTATGAGCCAATG			
QYr.inra-2DS	*Xgwm539*		CTGCTCTAAGATTCATGCAACC		17.34	Mallard et al 2005
			GAGGCTTGTGCCCTCTGTAG			
	Xgwm102		TCTCCCATCCAACGCCTC		8.06	
			TGTTGGTGGCTTGACTATTG			
QYr.inra-5BL.1	*Xgwm639c*		CTCTCTCCATTCGGTTTTCC		3.8	Mallard et al 2005
	Xgwm639a		CATGCCCCCCTTTTCTG		3.1	
QYr.inra-5BL.2	*Xgwm604*		TATATAGTTCAATATGACCCG		5.15	Mallard et al 2005
			ATCTTTTGAACCAAATGTG			
	Xgwm234a		GAGTCCTGATGTGAAGCTGTTG		5.35	
			CTCATTGGGGTGTGTACGTG			
QYrst.wgp-6BS.1	*Xbarc136*		GCGAGCTCACTGCACACTTACCC		3.9	Santra et al

			GCAACGCACCTTGATAATC			2008
	Xbarc101		GCTCCTCTCACGATCACGCAAAG			
			GCGAGTCGATCACACTATGAGCCAATG			
QYrst.wgp-6BS.2	*Xbarc136*		GCGAGCTCACTGCACACTTACCC		17.5	Santra et al 2008
			GCAACGCACCTTGATAATC			
	Xbarc101		GCTCCTCTCACGATCACGCAAAG			
			GCGAGTCGATCACACTATGAGCCAATG			
QYrtb.pau-5A	*Xbarc151*		TGAGGAAAATGTCTCTATAGCATCC		8.9	Chhuneja et al. 2008
			CGCATAAACACCTTCGCTCTTCCACTC			
	Xcfd12		GTTACCCAAACCTGCCCTTT	192		
			CTACGAGTCGGGATCAGCAT			
QYrtm.pau-2A	*Xwmc407*		GGTAATTCTAGGCTGACATATGCTC	135	3.6	Chhuneja et al. 2008
			CATATTTCCAAATCCCCAACTC			
	Xwmc170		ACATCCACGTTTATGTTGTTGC	230		
			TTGGTTGCTCAACGTTTACTTC			

CHAPTER TWO

Molecular Mapping of a Gene for Resistance to Stripe Rust in Spring Wheat Cultivar IDO377s

INTRODUCTION

Stripe rust (also known as yellow rust) is a disease of wheat, barley, rye, and 59 additional grass species (Line 2002). Wheat stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., has been reported in over 60 countries (Chen 2005). Unlike leaf rust and stem rust, stripe rust does not have any known alternate hosts for basidiospores to infect. Although fungicides are effective to stripe rust control since the first use in North America in 1981 (Line 2002), the application of fungicides adds much extra cost to wheat production. The best strategy to control stripe rust is growing resistant cultivars (Chen 2005).

Resistance to stripe rust can be generally separated into two categories: all-stage resistance (also called seedling resistance) and adult-plant resistance such as high temperature, adult-plant (HTAP) resistance. All-stage resistance is race-specific and can be detected at seedling stage but is also expressed at all growth stages (Chen 2005). Cultivars with all-stage resistance become susceptible very soon after they have been released. This is because all-stage resistance is often conferred by single genes. Evidence showed that rapid development of new virulent races of the pathogen from mutation and somatic recombination circumvented the all-stage resistance (Wellings and McIntosh 1990, Line and Qayoum 1992). In contrast, HTAP resistance is non-race specific, durable, and often quantitatively inherited (Qayoum and Line 1985; Chen and Line 1995a, 1995b; Line 2002; Chen 2005). More than 70 stripe rust resistance genes, designated *Yr* for yellow rust, have been identified in wheat (reviewed by Chen 2005). Molecular markers have been developed for numerous stripe rust resistance genes or quantitative trait loci (QTL) and have been used in marker-assisted

selection for developing resistant cultivars. New genes are still needed for developing resistant cultivars and for characterizing changes in virulence in populations of the stripe rust pathogen.

'IDO377s' (PI 591045), a hard white spring wheat cultivar developed from the cross Chova / 59Ab10293-5 = Gallo / Yecora reselection / 3 / Aurora / / Kalyansona / Bluebird / 4 /Norin 10/ Brevor / / Baart / Onas (http://www.laughlintrading.com/extended%20variety%20descriptions/377s.htm), was released in 1994 by the southern Idaho breeding program. The cultivar has been grown in the Pacific Northwest and used in breeding programs. Testing of IDO377s in the greenhouse with selected races and fields under natural infections of stripe rust at multiple locations for many years shows that the cultivar is resistant to the most of the races of *P. striiformis* f. sp. *tritici* (Chen et al., unpublished data). IDO377s was highly resistant to stripe rust until 2005 when it first became infected. The IDO377s crops have not been severely damaged in the field because the cultivar is still resistant to the predominant races and races virulent on IDO377s have been in very low frequency for the last three years (Chen, unpublished data).

Identifying and mapping the resistance gene(s) in IDO377s would be useful in developing stripe rust resistance cultivars and in characterizing new virulent races in the pathogen. The objectives of this study were to 1) determine the genetics of the stripe rust resistance in IDO377s, 2) identify molecular markers closely linked to the resistance gene(s) and determine its relationship with previously reported *Yr* genes, and 3) determine the usefulness of the markers in marker-assisted selection.

MATERIALS AND METHODS

Plant materials

The hard white spring wheat cultivar IDO377s was crossed with Australian spring

wheat genotype 'Avocet Susceptible' (AVS). IDO377s confers a high level of resistance to most races of *P. striiformis* f. sp. *tritici*, whereas AVS is susceptible to most races of *P. striiformis* f. sp. *tritici*. The seeds of AVS were kindly provided by Dr. Colin Wellings in the Plant Breeding Institute, University of Sydney. Three to five F_1 seeds from a single crossed head were planted in the greenhouse for selfing to produce F_2 seeds. Around 120 F_2 seeds from a single F_1 plant were planted in the greenhouse to get 114 F_3 lines. The parents and F_1, F_2, and F_3 progeny were used in seedling tests in the greenhouse to determine the inheritance pattern for all-stage resistance to stripe rust. Leaves of each parent and the 114 F_3 lines were collected for DNA extraction. A total of 108 wheat breeding lines and cultivars with or without IDO377s in their pedigrees, most of which were kindly provided by Dr. Kim Kidwell and Gary Shelton at Washington State University, were used to validate the flanking markers and determine polymorphisms of the marker loci in various wheat genotypes.

Nulli-tetrasomic (Sears 1966) and ditelosomic (Sears and Sears 1978) lines of 'Chinese Spring' wheat were used to localize RGAP markers to chromosome and sub-chromosome regions.

Pathogen materials

Eleven *P. striiformis* f. sp. *tritici* races (PST-1, PST-3, PST-7, PST-17, PST-21, PST-23, PST-43, PST-45, PST-59, PST-81, and PST-98) (Table 1), respectively various combinations of pathogen virulence (Chen 2005), were chosen to test seedlings of IDO377s and AVS. More recently identified races, such as PST-100, PST-114, PST-115, PST-116, and PST-127 were used to determine which races IDO377s was still resistant to and which races IDO377s has become susceptible to races PST-43 and PST-45, which were avirulent on IDO377s but virulent on AVS were selected to test seedlings of the F_1, F_2, and F_3 progenies.

Urediniospores of each isolate representing a specific race were increased on

susceptible genotypes and tested on the 20 wheat genotypes that are used to differentiate races of *P. striiformis* f. sp. *tritici* in the US (Chen et al. 2002; Chen 2005). This confirmed their purity before they were used for evaluation of stripe rust resistance of the parents and progeny of the AVS x IDO377s cross and the 108 wheat breeding lines and cultivars.

Evaluation of stripe rust resistance phenotype

Seeds of the two parents and F_1, F_2, and F_3 progenies were planted in plastic pots (5x5x5 cm) filled with a potting mixture of 6 peat moss : 2 perlite : 3 sand : 3 potting soil mix : 4 vermiculite with lime, Osmocote 14-14-14, and ammonium nitrate added at 1.7, 3.3, and 2.2 g/L, respectively. About 15 seeds for each parent, 3 seeds for F_1, 230 seeds for F_2, and 15 seeds for each of the 114 F_3 lines were planted with about 15 seeds in each pot except for the F_1. Seedlings at the two-leaf stage (about 10 days after planting) grown in a rust-free greenhouse (diurnal temperature cycle gradually changing from 10^{o}C at 2:00 am to 25^{o}C at 2:00 pm with the 16 h light/8 h dark cycle) were uniformly dusted with a mixture of urediniospores of a selected *P. striiformis* f. sp. *tritici* race with talc at a ratio of approximately 1:20. After inoculation, plants were placed in a dew chamber at 10^{o}C for 24 h and then transferred to a growth chamber operating at 16 h light and 8 h dark with diurnal temperatures gradually changing from 4^{o}C at 2:00 am to 20^{o}C at 2:00 pm (Chen and Line 1992a, 1992b). A set of 20 wheat genotypes used to differentiate races of *P. striiformis* f. sp. *tritici* were included in the tests to confirm the race identity. Infection type (IT) data were recorded 18-21 days after inoculation based on a 0-9 scale (Line and Qayoum 1992). Infection types 0-3, 4-6, and 7-9 were considered resistant, intermediate, and susceptible, respectively.

The 108 wheat breeding lines that have IDO377s in their pedigree were tested with PST-45 and PST-43 in the same way as described above.

DNA extraction

Genomic DNA was extracted from 15 plants for each of the parents and F_3 lines of the AVS/IDO377s cross and the 108 breeding lines using the methods described by Riede and Anderson (1996). Three grams of fresh leaves were ground in liquid nitrogen. Leaf tissue was ground with a glass rod and was mixed with 700 µl extraction buffer. After 30-min incubation at 65°C, 700 µl solution of 24:1 (v/v) chloroform/isoamyl alcohol was added, and the tube contents were vortexed thoroughly. Centrifugation was performed at 10,000 rpm for 12 min, and 600 µl of the upper phase solution was transferred to another 1.5-ml microcentrifuge tube. The DNA was precipitated with 1 ml of cold 95% ethanol (-20°C) and rinsed with 1 ml of 70% ethanol. The air-dried DNA was dissolved in 100 µl TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), and stored at -20°C. DNA was quantified using the mini-gel method (Maniatis et al. 1982) and spectrophotometer (NanoDrop ND-1000). The DNA stock solution was adjusted to 30 ng/µl with sterilized deionized and distilled water for use as working solution for polymerase chain reaction (PCR).

Bulk segregant analysis and testing markers with the F_3 population

Based on the phenotypic data, aliquots of DNA from ten F_3 lines exhibiting resistance in all plants and from ten F_3 lines exhibiting susceptibility in all plants were combined for making resistant and susceptible bulks, respectively. Segregating fragments specific to IDO377s and the resistant bulk or to AVS and the susceptible bulk were tested in a sub-population of 20 individual F_3 lines. Bands that were associated with the phenotypic disease data were tested further in the remaining 94 of the 114 F_3 lines. The segregation data of RGAP and SSR markers and the disease data obtained from the F_3 population were used to estimate the genetic linkage between the markers and the resistance locus.

RGAP and SSR Analyses

The RGAP method described previously (Chen et al. 1998; Shi et al. 2000) was used to screen for potential markers linked to the resistance locus. RGA primers were randomly paired with each other and first screened on the parents and the two bulks (Table 1). PCR reactions were performed in a GeneAmp® PCR System 9700 thermo-cycler. A 15 µl reaction mixture consisting of 36 ng of template DNA, 1.5 µl Mg-free 10X PCR buffer (Promega, Madison, WI, USA), 0.6 unit of Taq DNA polymerase (Promega), 7.5 mM of $MgCl_2$, 3 mM each of dATP, dCTP, dGTP, and dTTP (Sigma Chemical Co., St. Louis, MO, USA), and 36 ng of each primer synthesized by Operon Biotechnologies, Inc. (Huntsville, AL, USA). After 5 min of denaturation at 94°C, amplifications were programmed for 40 cycles, each consisting of 1 min at 94°C, 1 min at either 45, 50, 55, or 60°C (45°C for RGA primers; 50, 55, or 60°C for SSR primers depending on primer pair), 2 min at 72°C and followed by a 7 min extension step at 72°C. After PCR amplification, 6 µl of formamide loading buffer [98% formamide, 10 mM EDTA (pH 8.0), 0.5% (W/V) xylene cyanol, and 0.5% (W/V) bromophenol blue was added to the PCR products followed by 4 min denaturation at 94°C for electrophoresis in 5% polyacrylamide gels. After electrophoresis, gels were silver-stained according to the recommendation of the manufacturer (Promega, Madison, WI) and dried overnight and digitally scanned. Primer pairs showing specific bands to both IDO377s and the resistant bulks, or AVS and the susceptible bulks were used to genotype the F_3 population. Polymorphic markers tested with all 114 F_3 lines were scored and used to construct linkage maps. RGAP markers were designated using the *Xwgp* series (Shi et al. 2001).

SSR markers used in this study were selected from wheat chromosome 2B (Röder et al., 1998). The primer sequence information of SSR markers from chromosome 2B tested in this study was obtained from the GrainGenes 2.0 website (http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker). Primers of

Yr5STS7/8 marker were developed by Chen et al. (2003).

Determination of the chromosomal location of the IDO377s resistance gene

The complete set of the 21 Chinese Spring (CS) nulli-tetrasomic lines (N1AT1B, N1BT1A, N1DT1A, N2AT2D, N2BT2D, N2DT2A, N3AT3D, N3BT3D, N3DT3B, N4AT4D, N4BT4D, N4DT4B, N5AT5D, N5BT5A, N5DT5B, N6AT6D, N6BT6D, N6DT6A, N7AT7B, N7BT7A, and N7DT7B) (Sears 1966) were tested with two selected RGAP markers linked to the resistance locus to determine which chromosome the markers and the gene located to. After the resistance locus was determined to lie on chromosome 2B, the 2BL CS ditelosomic line was tested with the same markers to determine the chromosomal arm location. The 2BS ditelosomic line was not available. The chromosomal location of the resistance locus and RGAP markers were confirmed with polymorphic SSR markers specific to the chromosome arm.

40 SSR markers of known map locations on chromosome 2BL were tested for polymorphism with genomic DNA from the parents and the F_3 lines to confirm chromosomal locations of the resistance gene and identify more markers.

Data analyses

Chi-square tests were used to determine the goodness of fit of the observed numbers of plants or lines to the predicted segregation ratios of the F_2 and F_3 progenies to establish the number of stripe rust resistance genes, mode of inheritance, and relationships of genes for resistance to different races. A linkage map was constructed using the MAPMAKER program (Lander et al. 1987). Map distance in centi Morgans was calculated according to the Kosambi mapping function (Kosambi 1944). Chi-square tests were also used to determine the goodness of fit to a single-locus model for each marker in the F_3 population. If the probability

value is smaller than or equal to 0.05, the model will be rejected.

RESULTS

Phenotypical and genetic characterizations of stripe rust resistance in the AVS/IDO377s cross

The seedling IT (infection type) data of IDO377s and AVS inoculated with races PST-1, PST-3, PST-7, PST-17, PST-21, PST-23, PST-43, PST-45, PST-59, PST-81, PST-98, PST-100, PST-114, PST-115, PST-116, and PST-127 of *P. striiformis* f. sp. *triitici* are shown in Table 2. IDO377s was resistant to all tested races except PST-116 and PST-127 whereas AVS was susceptible to all tested races. PST-116 produced IT 5 on IDO377s, an intermediate reaction with intermediate sporulation and necrosis. PST-127, originally collected from IDO377s in the field in 2007, produced IT 9, a highly susceptible reaction.

The numbers of resistant (ITs 0, 1, or 2) and susceptible (ITs 7, 8, or 9) plants in F_2 and lines in the F_3 population inoculated with PST-43 and PST-45 and genetic analysis are shown in Table 3. When tested with PST-43, three tested F_1 plants had a resistant reaction (IT 2). F_2 plants segregated in a 3:1 ratio (*P-value* = 0.99) for resistant and susceptible plants, indicating that a single dominant gene in IDO377s confers resistance to PST-43. The 1:2:1 F_3 segregation ratio (*P-value* = 0.85) for homozygous resistance: segregating: homozygous susceptible lines confirmed the one-gene model. The same results were obtained with F_1, F_2, and F_3 progenies when tested with PST-45 (Table 3). Each of the F_3 lines had the same reaction to PST-43 and PST-45 and no recombinant lines were detected between reactions to these races, indicating the same gene controlling resistance to both races.

When tested with race PST-127 under both high (diurnal temperatures gradually changing from 10°C at 2:00 am to 35°C at 2:00 pm) and low (diurnal temperatures gradually

changing from 4°C at 2:00 am to 20°C at 2:00 pm) temperatures, AVS and IDO377s were susceptible (IT 7-8) at both seedling and adult-plant stages. These data indicated that IDO377s did not have HTAP resistance.

RGAP markers associated with the *YrIDO377s* locus

Of a total of 116 primer pairs from random combinations of 48 RGA primers screened in the bulk segregant analysis, 38 primers produced 40 amplicons that differentiated the susceptible parent (SP) and the susceptible bulk (SB) from the resistant parent (RP) and resistant bulk (RB). Eight primer pairs that generated eight strong and repeatable polymorphic bands were selected to test individual F_3 lines. The sequences of the 12 RGA primers producing the 8 polymorphic markers are shown in Table 2. As examples, Fig. 1 shows marker *Xwgp103* in the bulk segregant analysis of the RP, RB, SB, and SP, and Fig. 2 shows the banding pattern of F_3 lines screened with RGA primers RLRR For and NLRR Rev for marker *Xwgp99*. All eight RGAP markers were dominant and their designations, primers, sizes, presence and absence in IDO377s, AVS, and CS, and the *P* values for goodness of fit to a single locus are shown in Table 3. The relatively high *P* values indicated that these markers are reliable for constructing a linkage group for the IDO377s resistance gene.

Mapping the resistance gene to a wheat chromosome

To determine the chromosomal location of the resistance gene, the eight RGAP markers, which were present in RP and RB but not in SP and SB, were tested with CS and its 21 nulli-tetrasomic lines. RGAP markers *Xwgp99* and *Xwgp103* presented in CS were used to test the 21 nulli-tetrasomic lines. The unique bands were detected in all lines except N2BT2D (Fig. 3). The results indicated that the RGAP markers and the linked resistance gene were located on wheat chromosome 2B.

Only the 2BL CS ditelosomic line was then tested with the two RGAP markers as 2BS was not available. Both markers were detected in 2BL and thus, the resistance gene was located on the long arm of chromosome 2B.

Confirmation of the chromosomal location of *YrIDO377s* with SSR and STS markers

To identify more markers and to further confirm the chromosomal location of the resistance gene, a total of 20 SSR markers covering the long arm of chromosome 2B were screened among the two parents and the two bulks. Two markers, *Xwms501* amplified by the WMS501 primers (F: 5'-GGCTATCTCTGGCGCTAAAA-3' and R: 5'-TCCACAAACAAGTAGCGCC-3') (Röder et al. 1998) and *Xbarc139* amplified by the BARC139 primers (F: 5'-AGAAGCTCCCCTAAACTGAG-3' and R: 5'-CGACGCTGATGAATGAAT-3') (Liu and Anderson 2003), produced bands specific to both resistant parent and resistant bulk or both susceptible parent and the susceptible bulk. *Xwms501* was a co-dominant marker producing a 166-bp band in IDO377s and a 172-bp band in AVS. *Xbarc139* was a dominant marker (156 bp) presented in IDO377s. Linkage analysis using the two markers with the 114 F_3 lines indicated that they were linked to the resistant gene in IDO377s. As an example, the pattern of marker *Xwms501* in a polyacrylamide gel tested with the F_3 lines is shown in Fig. 4. The two SSR markers associated with the long-arm of chromosome 2B also further confirmed that the resistance gene in IDO377s is on the long arm of chromosome 2B.

To determine the relationship between *YrIDO377s* and *Yr5*, a reported stripe rust resistance gene located on the long arm of chromosome 2B, a STS marker, *Yr5STS7/8* that is amplified by primers Yr5STS7 (5'- GTGTACAATTCACCTAGAG-3') and Yr5STS8 (5'-GCAAGTTTTCTCCCTAT-3') and closely linked to the *Yr5* stripe rust resistance gene (Chen et al. 2003), was used to test the F_3 progeny lines. The co-dominant *Yr5STS7/8* STS

marker produced polymorphic bands that were specific to both resistant parent and resistant bulk or both susceptible parent and the susceptible bulk. Linkage analysis using the *Yr5STS7* marker with the 114 F_3 lines indicated that it was linked to *YrIDO377s*. These results further confirmed the chromosomal location of *YrIDO377s* on the long arm of chromosome 2B.

Construction of a linkage group for *YrIDO377s*

A linkage group containing *YrIDO377s* (Fig. 5) was estimated with 8 RGAP markers, 2 SSR markers, and 1 STS marker tested with the 114 F_3 lines. The *Xwms501* SSR marker mapped another stripe rust resistance gene, *YrZak,* indicating it mapped on chromosome 2BL (X. X. Sui and X. M. Chen unpublished data). The *Yr5STS7/8* STS marker put the *Yr5* resistance gene to the linkage group. The two closest flanking RGAP markers, *Xwgp99* and *Xwgp103*, were linked to *YrIDO377s* with genetic distances of 4.4 and 5.5 cM, respectively. The closest SSR marker, *Xwms501*, was linked to the gene with a genetic distance of 16.0 cM. This marker indicated that *YrZak* is 12.0 cM away from *YrIDO377s*. The *Yr5STS7/8* STS marker had a genetic distance of 38.6 cM from the *YrIDO377s* locus. This data indicated that *YrIDO377s*, *YrZak*, and *Yr5* were at different loci.

Comparison of *YrIDO377s* to other *Yr* genes on 2BL using various races

So far, the *Yr* genes that have been reported on chromosome 2BL are *Yr5* and *Yr7* (Chen 2005). Recently, *YrZak* was found on long arm of chromosome 2B by Xinxia Sui (personal communication). To determine the relationships of *YrIDO377s* with these genes, IDO377s was tested together with wheat genotypes AVS/6*Yr5 (*Yr5*), Lee (*Yr7*), and Zak (*YrZak*) with races PST-17, PST-59, PST-100, PST-116, and PST-127. The reactions of the wheat genotypes to these races are shown in Table 5. *Yr5* was effective against all five races; *Yr7* was ineffective against all five races; *YrZak* was effective to PST-17 and PST-59, but

ineffective to PST-100, PST-116, and PST-127; while *YrIDO377s* was resistant to PST-17, PST-59, and PST-100, intermediately resistant to PST-116, and highly susceptible to PST-127. The rust testing data, together with the linkage mapping data, indicated that *YrIDO377s* was different from other genes mapped to 2BL.

Three crosses between AVS/6*Yr5, Zak, and IDO377s were made in the greenhouse and F_1 seeds from each cross were planted for F_2 seeds. F_2 plants will be tested to further determine the genetic distances between *YrIDO377s* and *Yr5*, *Yr7*, or *YrZak*.

The usefulness of the flanking markers in marker-assisted selection

A total of 108 cultivars and breeding lines with IDO377s or related genotypes in their pedigrees were tested with races PST-43, PST-45, and PST-127 and the two flanking markers *Xwgp99* and *Xwgp103* (Table 5). When tested with PST-43 or PST-45, which produced the same reactions data, 90 of 108 lines (83.3%) were resistant to PST-43 and PST-45, but susceptible to PST-127, the same reactions as IDO377s to these races, and therefore, they could be considered to have *YrIDO377s*. The remaining 18 lines (16.7%) were susceptible to the three races and therefore, could be considered not to have the gene. In the group of 90 lines potentially carrying *YrIDO377s* based on these tests, 76 lines (84.4%) had both markers *Xwgp99* and *Xwgp103*; 4 lines (4.4%) had only *Xwgp99*; 9 lines had only *Xwgp103*; and only 1 line (1.1%) did not have any of the markers. Of the 18 lines potentially without *YrIDO377s* based on the race reactions, 13 lines (72.2%) did not have any of the markers; 2 lines (11.1%) had only *Xwgp99*; 3 lines (16.7%) had only *Xwgp103*; and none of the lines had both the markers. When the two groups were combined, 89 lines (82.4%) had marker genotypes that completely agreed with the reaction phenotypes, only 1 line (0.9%) had a marker type not matching the reaction phenotype, and 18 lines (16.7%) had either one of the markers that partially matching with the reaction phenotypes. These results indicated that the flanking

markers were highly associated with the *YrIDO377s* resistance.

DISCUSSION

Genetically, the molecular markers identified in this study were linked to *YrIDO377s* and other resistance genes on long arm of wheat chromosome 2B. The linkage data shed light on the relationship between *Yr5*, *YrZak*, and *YrIDO377s*. Combined the linkage groups constructed in the three crosses (AVS/Zak, AVS/Yr5, AVS/IDO377s), *YrZak* is 12.0 cM of genetic distance away from the *YrIDO377s*, while the *Yr5* gene is linked to the *YrIDO377s* gene with a genetic distance of 39.3 cM. The F_2 progeny test of the Yr5/Zak cross was consistent with the genetic distance generated by the marker linkage analysis (Data not shown). Although the results of progeny segregation tests of cross Yr5/IDO377s and Zak/IDO377s have not completed, we are convinced by the rust testing data (Table 4), together with the linkage mapping data that *YrIDO377s* is a novel gene different from all previously reported gene.

Stripe rust has been a major problem in wheat production in the United States (Chen 2005, 2007). Breeding resistant cultivars to control the disease is the most efficient and economical approach. Because new races of *P. striiformis* can overcome race-specific resistance in cultivars, more resistance genes are needed for breeding programs. The race-specific, all-stage resistance that is conferred mostly by single dominant genes has high level of resistance, but is easily overcome by new races of the pathogen (Line and Qayoum 1992; Chen 2005, 2007). The hard white spring cultivar IDO377s used in this study was resistant to all races in the Pacific Northwest before 2005 but became susceptible to races PST-116 and PST-127, which appeared after 2005. Although HTAP resistance is durable and non race-specific, incorporating HTAP resistance into commercial cultivars is more difficult because it is partial, often controlled by multiple quantitative trait loci, and under the

influence of plant growth stage and temperature (Line and Chen 1995, Chen 2005). Therefore, a better strategy for breeding resistant cultivars is to combine all-stage resistance with HTAP resistance.

In spite of losing resistance to stripe rust after 2005, IDO377s are still being grown and used in breeding programs because it has many other desirable traits. The markers identified in this study can be efficiently used by wheat breeders to detect *YrIDO377s* in breeding lines and cultivars. The two flanking markers *Xwgp99* and *Xwgp103* identified in this study showed high reliability for tagging *YrIDO377s* by testing 108 cultivars and breeding lines. Two spring wheat cultivars Lolo (PI 614840) and Otis (PI 634866), the former was released by the southern Idaho breeding program in 2000 (Souza et al. 2000) and the latter was released by the Washington State University spring wheat breeding program in 2005 (Kidwell et al. 2005) were included in this study because they were developed using IDO377s. Both the race reaction and molecular data showed that Lolo has *YrIDO377s* and Otis does not have the gene. These results agreed with our many-year field and greenhouse testing data for stripe rust resistance (Chen, unpublished data). Interestingly, the mismatch by *Xwgp99* to the race reaction phenotype was only 6 out of 108 lines (5.6%) and that by *Xwgp103* was 11 out of 108 lines (10.2%). When these mismatch rates were treated as frequencies of recombination between the gene and the markers, the data correlated well with the genetic distances, 4.4 and 5.5 cM between *YrIDO377s* and *Xwgp99* and between the gene and *Xwgp103*, respectively. The results demonstrated the usefulness of association mapping, an approach that does not involve making crosses and is becoming popular in molecular mapping (Cockram et al. 2008).

The RGAP technique used in this study helped quickly identify resistance-gene linked markers in the bulk segregant analysis. More resistance genes in plants have been identified and cloned since the RGAP technique established in 1998 (Shi et al. 2001, Yan et al. 2003,

2007, 2008, Lin and Chen 2007, 2008). New RGA primers can be developed to improve the efficiency and power of the strategy by utilizing the recent report of nucleotide binding site-leucine rich repeat-encoding and related genes in other plant species (Tan et al. 2007).

The utilization of nulli-tetrasomic lines, ditelosomic lines, and deletion lines of Chinese Spring speeded up mapping of *YrIDO377s* to the long arm of chromosome 2B. Screening 2BL specific SSR markers allowed us to confirm the chromosomal location and mapping the resistance gene to a smaller region of the chromosome. Similar to previous studies, the present study proved this mapping strategy efficient.

In conclusion, this study identified a novel gene in wheat for resistance to stripe rust. Because the *YrIDO377s* resistance is race-specific, it should be used in combination with other genes or QTL for either all-stage resistance or durable HTAP resistance. The flanking markers, *Xwgp99* and *Xwgp103*, should be useful for combining *YrIDO377s* with other resistance genes for durable resistance. Because these RGAP markers need to be run in polyacrylamide gels, which are relatively time-consuming and complicated, more user-friendly STS markers can be developed from the flanking markers for use of agarose gel electrophoresis to tag the resistance gene in breeding lines.

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TABLE 1. Infection types of IDO377s and Avocet Susceptible (AVS) to races of *Puccinia striiformis* f. sp. *tritici* tested in the seedling stage under controlled greenhouse conditions

		Infection Type	
PST race[a]	Virulence formula	AVS	IDO377s
PST-1	1, 2	9	2
PST-3	1, 3	9	2
PST-7	1, 3, 5	9	2
PST-17	1, 2, 3, 9, 11	9	2
PST-21	2	9	2
PST-23	1, 3, 6, 9, 10	9	2
PST-43	1, 3, 4, 5, 12, 14	9	2
PST-45	1, 3, 12, 13, 15	9	2
PST-59	1, 3, 11, 12, 16	9	2
PST-81	1, 14	9	2
PST-98	1, 3, 8, 10, 11, 12, 16, 17, 18, 19, 20	9	2
PST-100	1, 3, 8, 9, 10, 11, 12,16, 17, 18, 19, 20	9	2
PST-111	1, 3, 5, 8, 10 , 11, 12, 16 , 17, 18, 19, 20	9	2
PST-116	1, 3, 4, 5, 8, 9, 10, 11, 12, 14, 16 , 17, 18, 19, 20	9	5
PST-127	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20	9	9

[a] Races PST-1 to PST-116 were previously described by Line and Qayoum (1992), Chen et al. (2002), and Chen (2005, 2007). Race PST-127 is a new race that was collected from IDO377s in the field near Pullman, WA and identified in 2007 during this study.

TABLE 2. Resistance gene analog (RGA) primers used to identify resistance gene analog polymorphism (RGAP) markers for mapping the gene in IDO377s for resistance to races of *Puccinia striiformis* f. sp. *tritici*

RGA primers[a]	Sequence (5'-3')	Gene	Domain	references
CLRR-INV2	TCTTCAGCTATCTGC	*Cf9*	LRR	Yan et al. 2003
Cre3-k3	CTGCAGTAAGCAAAGCAACG	*Cre3*	Kinase	This study
Cre3LR-F	CACACACTCGTCAGTCTGCC	*Cre3*	LRR	Yan and Chen 2007
NLRR Rev	TATAAAAAGTGCCGGACT	*N*	LRR	Chen et al. 1998a
NPLOOP	TCAATTAATGTTTGAGTTATTG TA	*N*	P-loop	This study
Ptokin1	GCATTGGAACAAGGTGAA	*Pto*	Kinase	Chen et al. 1998a
RLRR For	CGCAACCACTAGAGTAAC	*Rps2*	LRR	Chen et al. 1998a
RLRR Rev	ACACTGGTCCATGAGGTT	*Rps2*	LRR	Chen et al. 1998a
S1	GGTGGGGTTGGGAAGACAACG	*L6, N, Rps2*	NBS	This study
S2	GGIGGIGTIGGIAAIACIAC	*N, Rps2*	P-loop	Leister et al. 1996
S2-INV	CAICAIAAIGGITGIGGIGG	*N, Rps2*	P-loop	Pahalawatta and Chen 2005a
XLRR For	CCGTTGGACAGGAAGGAG	*Xa21*	LRR	Chen et al. 1998a

TABLE 3. Primer pairs, probability (*P*) values for a 3:1 segregation ratio in the F_3 progeny, and sizes of markers used for mapping the *YrIDO377s* gene conferring resistance to *Puccinia striiformis* f. sp. *tritici* and their presence or absence in the resistant parent IDO377s, susceptible parent AVS, and Chinese spring (CS)

Markers	Primers	No. of Present marker	No. of Absence marker	*P* for a 3:1 ratio	Size (bp)[a]	Presence or absence[b]		
						IDO377s	AVS	CS
RGAP								
Xwgp99	RLRR For/NLRR Rev	88	28	0.83	1000	+	-	+
Xwgp100	Pto kin1/S1	92	24	0.28	800	+	-	-
Xwgp101	Cre3-k3/Cre3LR-F	85	31	0.67	480	+	-	+
Xwgp102	XLRR For/Cre3LR-F	91	25	0.39	650	+	-	-
Xwgp103	RLRR For/S2	86	30	0.83	680	+	-	+
Xwgp104	RLRR Rev/S2-INV	89	27	0.67	580	+	-	-
Xwgp105	NPLOOP/S2-INV	90	26	0.52	680	+	-	-
Xwgp106	RLRR For/CLRR-INV2	86	30	0.83	550	+	-	+
SSR								
Xwms501	WMS501	89	27	0.67	166	+	-	-
Xwms501	WMS501	89	27	0.67	172	-	+	+
Xbarc139	BARC139	88	28	0.83	156	+	-	-
STS								
Yr5STS 7/8	Yr5STS7/8	86	30	0.83	480	+	-	+

[a] Sizes of all markers were estimated based on 1-kb plus DNA marker.

[b] CS = Chinese Spring, AVS = Avocet Susceptible, + = presence, and - = absence

TABLE 4. Segregation for seedling resistance to PST-43 and PST-45 in the F_2 and F_3 generations of AVS/IDO377s.

Race	Generation	Observed number of F_2 plants or F_3 lines			Expected ratio	χ^2	*P*
		Resistance	Segregating	Susceptible			
PST-43	F_2	226	NA[a]	74	3:1	0.02	0.99
	F_3	31	56	27	1:2:1	0.32	0.85
PST-45	F_2	223	NA	77	3:1	0.07	0.93
	F_3	31	56	27	1:2:1	0.32	0.85

[a] NA = not applicable

TABLE 5. Infection types of wheat genotypes with *Yr* genes on long arm of chromosome 2B to races of *Puccinia striiformis* f. sp. *tritici*

Yr gene	Wheat genotype	Infection type to races of *Puccinia* f. sp. *tritici*						
		17	43	45	59	100	116	127
Yr5	AVS/6*Yr5	2	2	2	2	2	2	2
Yr7	Lee	8	2 [a]	2 [a]	8	8	8	8
YrZak	Zak	2	2	2	2	8	8	8
YrIDO377s	IDO377s	2	2	2	2	2	6	8

[a] not virulent on Lee.

TABLE 6. Infection types produced by races PST-43 and PST-127 of *Puccinia striiformis* f. sp. *tritici* on and presence or absence of resistance gene analog polymorphism (RGAP) markers *Xwgp99* and *Xwgp103* in 108 spring wheat breeding lines

		Infection type[b] produced by race		Presence (+) and absence (-) of marker	
Name	Pedigree[a]	PST-43 or 45	PST-127	*Xwgp99*	*Xwgp103*
HW030002	**IDO377s**/IDO552	2	8	+	+
HW030003	**IDO377s**/CA1128	2	8	+	+
HW030004	**IDO377s**/CA1128	2	8	+	+
HW030005	**IDO377s**/IDO546	2	8	+	+
HW030006	**IDO377s**/OR920307	2	8	+	+
HW030007	**IDO377s**/OR920307	2	8	+	+
HW030008	**IDO377s**/OR920307	2	8	+	+
HW030009	**IDO377s**/S9700017	2	8	+	+
HW030010	**IDO377s**/S9700020	2	8	+	+
HW030011	**IDO377s**/S9700020	2	8	+	+
HW030012	**IDO377s**/S9700020	2	8	+	+

HW030013	**IDO377s**/S9700020	2	8	+	+
HW030014	**IDO377s**/S9700020	2	8	+	+
HW030017	**IDO377s**/HW00097	2	8	+	+
HW030019	**IDO377s**/HW00097	2	8	+	+
HW030020	**IDO377s**/HW00097	2	8	+	+
HW030021	**IDO377s**/HW00097	2	8	+	+
HW030071	WA7780/Aroona//**IDO377s**/3/IDO533	2	8	+	+
HW030074	WA7780/Aroona//**IDO377s**/3/IDO533	2	8	+	+
HW030075	WA7780/Aroona//**IDO377s**/3/IDO533	2	8	+	+
HW030076	WA7780/Aroona//**IDO377s**/3/IDO533	2	8	+	+
HW030078	WA7780/Aroona//**IDO377s**/3/IDO533	2	8	+	+
HW030079	WA7780/Aroona//**IDO377s**/3/IDO533	2	8	+	+
HW030080	WA7780/Aroona//**IDO377s**/3/IDO533	2	8	+	+
HW040168	MACON/OTIS 2003F5	2	8	+	+
HW040169	MACON/OTIS 2003F5	2	8	+	+
HW050243	**IDO377S**/906R short white	2	8	+	+

HW050245	**IDO377S**/SSII	2	8	+	+
HW050246	**IDO377S**/SSII	2	8	+	+
HW060022	Otis/Perouse (PHW)	2	8	+	+
HW060072	Macon/HWN990149T//Otis	2	8	+	+
HW060073	Macon/HWN990149T//Otis	2	8	+	+
HW060080	Macon /Otis//WA007945	2	8	+	+
HW060081	Macon /Otis//Lolo	2	8	+	+
HW060130	Otis /Janz//WA7930	2	8	+	+
HW060131	Otis /Janz //WA007945	2	8	+	+
HW060136	Otis /Pelsart //HWD2K0084	2	8	+	+
HW060138	Otis /Perouse//WA7930	2	8	+	+
HW060145	Otis /Perouse //HWD2K0084	2	8	+	+
HW060147	Otis /Perouse //WA007945	2	8	+	+
HW060150	Otis /Sunmist //WA007956	2	8	+	+
HW060152	Otis /Sunmist //HWD2K0084	2	8	+	+
HW060153	Otis /Sunmist //WA007945	2	8	+	+

HW060154	Otis /Sunstate//IDO594	2	8	+	+
HW060155	Otis /Sunstate//IDO594	2	8	+	+
HW060158	Otis /Sunstate //HWD2K0084	2	8	+	+
HW060159	Otis /Sunstate ?//WA007945	2	8	+	+
HW060173	Macon/Otis//BZ998-447W	2	8	+	+
HW060174	Macon/Otis//BZ998-447W	2	8	+	+
HW060175	Macon/Otis//BZ998-447W	2	8	+	+
HW060178	Macon/Otis//Lolo	2	8	+	+
06145/2-2	UC1107//**IDO377s**/UC1107 (resel from 06100/4)	2	8	+	+
Lolo (PI 614840, IDO533)	Oasis/**IDO377s**	2	8	+	+
A01206S-K-7	Lolo*3/Js-12-Mu-6	2	8	+	+
A99050S-J-1	Lolo//NK751/Oasis	2	8	+	+
A99454S-2	Lolo//Klasic*3/AmIDOon	2	8	+	+
A99463S-5	Lolo/MTHW9420	2	8	+	+

A03814S-L	[McNeal/Lolo]F3 seln//2*Lolo	2	8	+	+
A00112S-E-1	McNeal/IDO470//Lolo	2	8	+	+
A00561-2	Jerome/Lolo	2	8	+	+
A981146S-3B-1	Lolo/HJ98	2	8	+	+
A01585S-1A-1B-1	LoloMu/4*Lolo	2	8	+	+
A01585S-1A-1B-2	LoloMu/4*Lolo	2	8	+	+
ML455-533-2	ML455/IDO533(LOLO)	2	8	+	+
HW060003	Macon/Otis	8	8	-	-
HW060021	Otis/Pelsart (HW Fac)	8	8	-	-
HW060023	Otis/Perouse (PHW)	8	8	-	-
HW060071	Macon/HWN990149T//Otis	8	8	-	-
HW060079	Macon /Otis//HWD2K0084	8	8	-	-
HW060140	Otis /Perouse //BZ998-447W	8	8	-	-
HW060143	Otis /Perouse //BZ998-447W	8	8	-	-
HW060144	Otis /Perouse //HWD2K0084	8	8	-	-
HW060149	Otis /Sunmist //BZ998-447W	8	8	-	-

HW060177	Macon/Otis//Lolo	8	8	-	-
Otis (PI 634866, WA7931)	**IDO377s**/3/Tanager 'S'/Torim 73//Spillman	8	8	-	-
A99063S-K-2B-7	P29/IDO521//Lolo	8	8	-	-
A01585S-1A-1B-3	LoloMu/4*Lolo	8	8	-	-
HW030077	WA7780/Aroona//**IDO377s**/3/IDO533	2	8	+	-
HW050241	**IDO377S**/906R short white	2	8	+	-
HW050242	**IDO377S**/906R short white	2	8	+	-
HW030018	**IDO377s**/HW00097	2	8	-	+
HW030072	WA7780/Aroona//**IDO377s**/3/IDO533	2	8	-	+
HW030073	WA7780/Aroona//**IDO377s**/3/IDO533	2	8	-	+
HW060134	Otis /Pelsart //BZ998-447W	2	8	-	+
HW060135	Otis /Pelsart //BZ998-447W	2	8	-	+
A98073S-B-3B-1	Lolo*2//Mirlo/Buc	2	8	-	+
HW050252	OTIS RESELECT 2005 7+9 Hfly Resistant	8	8	+	-
HW060146	Otis /Perouse //HWD2K0084	8	8	+	-

HW060176	Macon/Otis//Lolo	8	8	-	+
HW060132	Otis /Pelsart//WA7930	8	8	-	+
HW060139	Otis /Perouse//WA7930	8	8	-	+
HW030015	**IDO377s**/S9700149	2, 8	8	+	+
HW030016	**IDO377s**/S9700405	2, 7	8	+	+
HW040170	MACON/OTIS 2003F5	2, 8	8	+	+
HW060133	Otis /Pelsart//IDO594	2, 8	8	+	+
HW060137	Otis /Pelsart //HWD2K0084	2, 8	8	+	+
HW060148	Otis /Sunmist//IDO594	2, 8	8	+	+
HW060151	Otis /Sunmist//WA007956	2, 8	8	+	+
HW060156	Otis /Sunstate//IDO594	2, 8	8	+	+
HW060157	Otis /Sunstate //WA007956	3, 8	8	+	+
A03814S-E	[McNeal/Lolo]F3 seln//2*Lolo	2, 8	8	+	+
A00078S-C-1	Conan/3/2*Cadoux//Wilgoyne/M2	2, 8	8	+	+
A00561-1	Jerome/Lolo	2, 8	8	+	+
HW060141	Otis /Perouse //BZ998-447W	2, 8	8	+	-

HW060142	Otis /Perouse //BZ998-447W	2, 8	8	-	+
06015/22	UC1107//**IDO377s**/UC1107	2, 8	8	-	+
A03814S-H	[McNeal/Lolo]F3 seln//2*Lolo	2, 8	8	-	+
IDO667	**IDO377s**/PH992313	2, 8	8	-	-

[a] IDO377s and IDO377s-related genotypes were marked in bold.

[b] Infection types were based on the 0 - 9 scale as described by Line and Qayoum (1992). One value indicated uniform and two values indicated segregating with the majority of plants with the first infection type value.

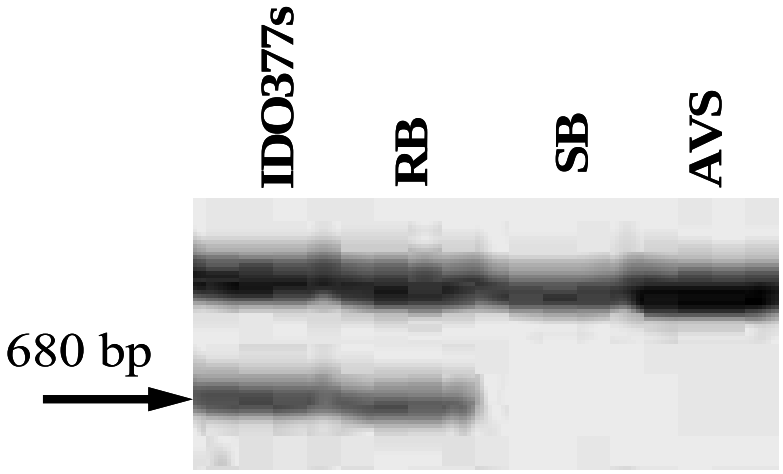


FIGURE 1. Silver-stained denaturing polyacrylamide gel showing the resistance gene analog polymorphism (RGAP) marker *Xwgp103* amplified with primers RLRR For (5'-CGCAACCACTAGAGTAAC-3') and S2 (5'-GGIGGIGTIGGIAAIACIAC-3') in bulk segregant analysis with the resistant parent IDO377s, resistant bulk (RB), susceptible bulk (SB), and susceptible parent Avocet Susceptible (AVS).

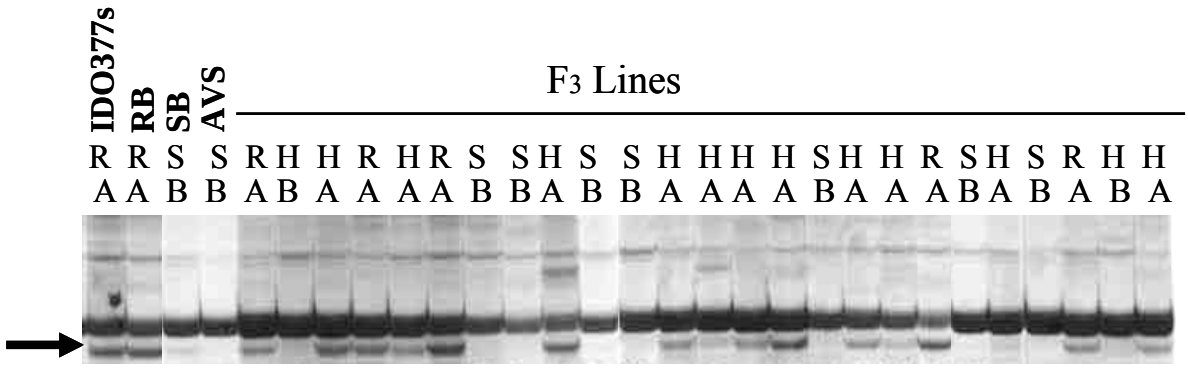
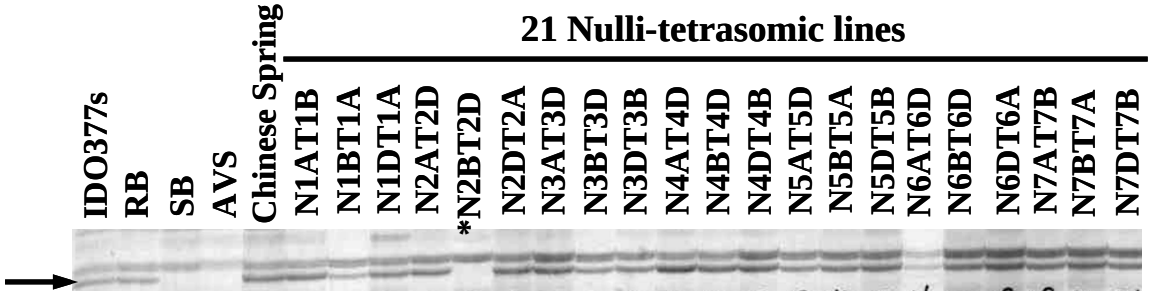


FIGURE 2. Silver-stained denaturing polyacrylamide gel showing the resistance gene analog polymorphism (RGAP) marker *Xwgp99* (arrow, 1000 bp) amplified with primers RLRR For (5'-CGCAACCACTAGAGTAAC-3') and NLRR Rev (5'-TATAAAAAGTGCCGGACT-3') in bulk segregant analysis of F_3 progeny. RP = resistant parent IDO377s, RB = resistant bulk, SB = susceptible bulk, and SP = susceptible parent Avocet Susceptible (AVS). For the stripe rust reaction phenotype, R = resistant, S = susceptible, and H = heterozygous. For the marker type, A = presence of the marker and B = absence of the marker.

A



B

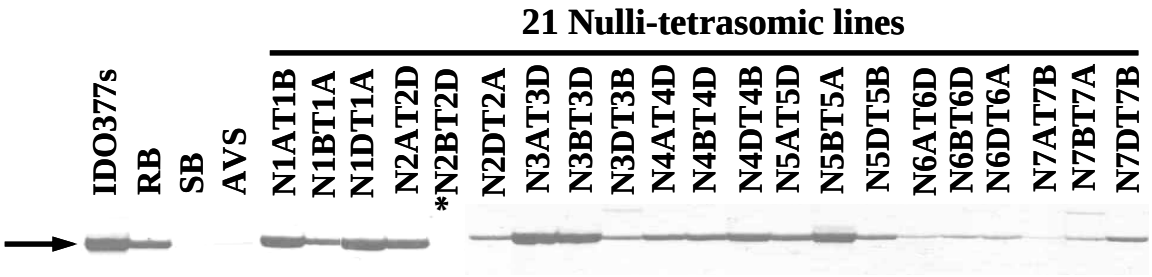


FIGURE 3. Silver stained denaturing polyacrylamide gels showing segregation patterns of RGAP markers *Xwgp99* (arrow, 1000 bp) amplified with primers RLRR For (5'-CGCAACCACTAGAGTAAC-3') and NLRR Rev (5'- TATAAAAAGTGCCGGACT-3') (**A**) and *Xwgp103* (arrow, 680 bp) amplified with primers RLRR For and S2 (5'-GGIGGIGTIGGIAAIACIAC-3') (**B**) and their association with 21 nulli-tetrasomic lines. RB - resistant bulk, SB - susceptible bulk.

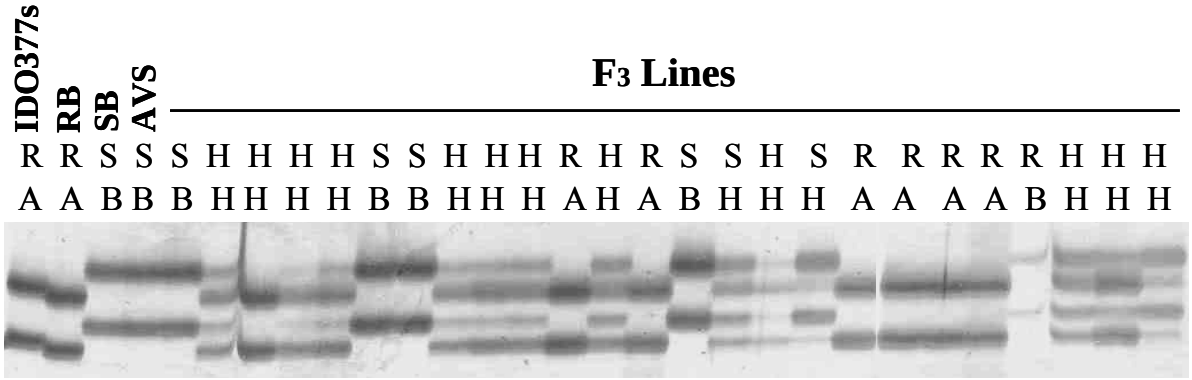


FIGURE 4. Silver-stained denaturing polyacrylamide gel showing the SSR marker *Xwms501* amplified with forward primer 5'-GGCTATCTCTGGCGCTAAAA-3' and reverse primer 5'-TCCACAAACAAGTAGCGCC-3' in the bulk segregant analysis of F_3 progeny. RP = resistant parent IDO377s, RB = resistant bulk, SB = susceptible bulk, and SP = susceptible parent Avocet Susceptible (AVS). For stripe rust reaction phenotypes, R indicates resistant, S indicates susceptible, H indicates segregating in F_3 and heterozygous in its F_2 parental plant, A-presence of marker A, B-presence of marker B, C-presence of both marker A and B.

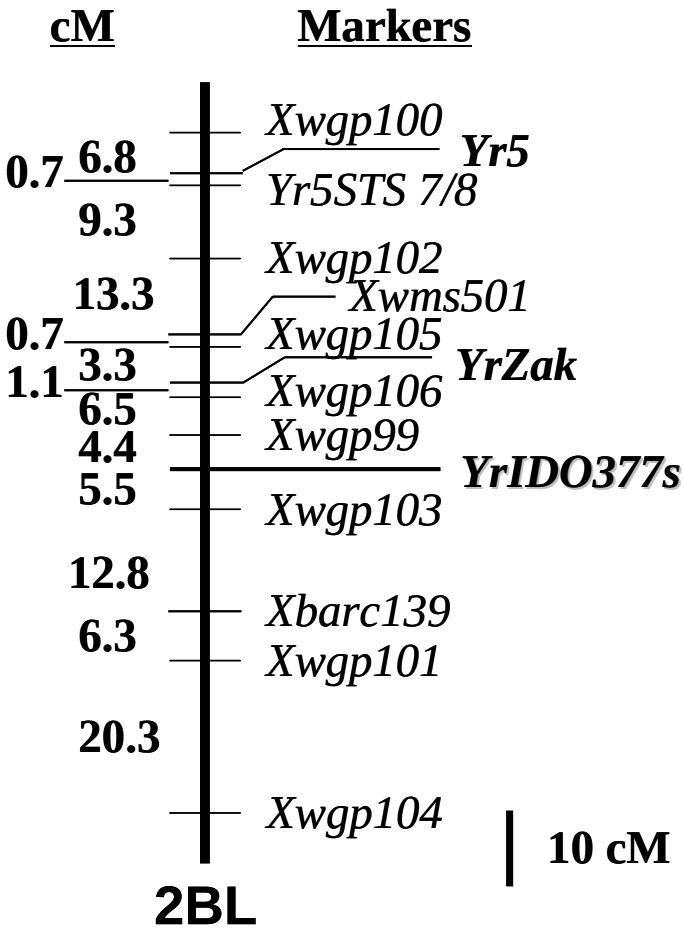


FIGURE 5. Linkage map for the resistance gene *YrIDO377s* constructed with 8 RGAP markers (*Xwgp99* to *Xwgp106*), 2 SSR markers (*Xwms501* and *Xbarc139*) and 1 STS marker (*Yr5STS7/8*). The markers linked to the resistance gene *YrIDO377s* are shown on the right. The linkage on the long arm of chromosome 2B was determined by two RGAP markers *Xwgp99* and *Xwgp103* using 21 nulli-tetrasomic Chinese Spring lines.

Chapter 3

Identification of a New Race of *Puccinia striiformis* f. sp. *tritici* Virulent on IDO377s

INTRODUCTION

In North America, wheat stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., was first recognized in 1915 (Carleton 1915). Race identification in North America began in the 1920s based on the host range and resistance of cultivars (Hungerford and Owens 1923). However, stripe rust disease had not caused much damage from mid-1930s to mid-1950s until a severe epidemic occurred in the late 1950s to the early 1960s. The race identification study stopped in the 1930s and restarted in the 1950s when new races became prevalent in North America (Line 2002). It was not until 1968, the first established race identification system constructed by Line and his colleagues had been widely applied (Line et al. 1970). Stripe rust has been reported widely spread to western states of Rocky Mountains, south-central states and the Great Plain since 2000(Chen et al. 2002, Chen 2005). The yield losses caused by stripe rust of wheat in the US were estimated to be 39.65, 88.88, and 73.28 million bushels in 2001, 2003, and 2005, respectively (Chen 2005, 2007; www.cdl.umn.edu/loss/html). In 2005, wheat stripe rust occurred in more than 30 states, which made it the recorded history of the US as the most widely distributed year (Chen 2007). Even though more emphasis has been put into breeding resistant cultivars to control the destructive disease, new races could circumvent resistant cultivars in a few years after they were grown (Purdy 1963). It is important to identify the races emerged every year and understand the changes of the pathogen virulence for documenting the distribution and severity of stripe rust and developing better disease management in the future.

Every year, rust program at Washington State University collects and receives stripe rust samples from experimental nurseries and commercial fields to monitor the distribution, prevalence, severity, and virulence of *P. striiformis* throughout the United States. A total of 126 races of *P. striiformis* f. sp. *tritici* had been identified in the US before 2007 (Line and Qayoum 1992; Line and Chen 1996; Chen et al. 2002; Chen 2005, 2007).

'IDO377s' (PI 591045), a hard white spring wheat cultivar, has been grown in the Pacific Northwest for many years since it was released in 1994. The cultivar had been highly resistant to stripe rust until recently when it became susceptible. Indentifying the isolates collected from IDO377s can help us better understand the virulence changes of *P. striiformis* f. sp. *tritici* and the interaction between the plant and the pathogen. The objective of this study was to determine the races of stripe rust collected from IDO377s.

MATERIALS AND METHODS

Plant materials and inoculation

A total of 20 wheat genotypes that are currently used to differentiate races of *P. striiformis* f. sp. *tritici* (Chen et al. 2002; Chen 2005) were used in this study. To identify races from stripe rust samples, differential genotypes were grown to the two-leaf stage (about 10 days after planting) in a rust-free greenhouse under diurnal temperature cycles gradually changing from 10°C at 2:00 am to 25°C at 2:00 pm with the 16 h light/8 h dark cycle. Plants were uniformly dusted with a mixture of urediniospores of a *P. striiformis* f. sp. *tritici* isolate to be tested with talc at a ratio of approximately 1:20. After inoculation, plants were placed in a dew chamber at 10°C for 24 h and then transferred to a growth chamber operating at 16 h light and 8 h dark with diurnal temperatures gradually changing from 4°C at 2:00 am to 20°C at 2:00 pm. Infection type (IT) data were recorded 18-21 days after inoculation based on a

0-9 scale (Line and Qayoum 1992).

Isolates of *P. striiformis* f. sp. *tritici* and race identification

Two isolates, named as 07-239 and 07-240, were collected from IDO377s in the Whitlow Farm at Pullman, Washington in July 2007. Isolates were first increased on wheat genotype 'Nugaines' that is susceptible in the seedling stage to all races of *P. striiformis* f. sp. *tritici* identified so far in the US to get enough urediniospores for further testing. Urediniospores of each isolate were then used to inoculate the 20 wheat differential genotypes to get the virulence patterns using the standard method as previously described (Line and Qayoum 1992; Chen et al. 2002; Chen 2005). To determine whether the new virulence patterns were produced by a single new race or a mixture of previously identified races, four differential cultivars Chinese 166 (*Yr1*), Druchamp (*Yr3a*, *YrDru1*, and *YrDru2*), Tyee (*YrTye*), and Hyak (*Yr17* and *YrTye*) were selected for obtaining subisolates because races with the combination of the virulences to the four differentials had not been previously detected.

Chinese 166, Druchamp, Tyee, and Hyak were planted into individual pots (two pots each; one for each sub-isolate) and inoculated with each of the original isolates. Each pot of inoculated plants was kept in a plastic booth to keep from cross contamination. Urediniospores from plants of each pot were collected and labeled with a sub-isolate identification number. Thus, sub-isolates 07-239-2, 07-239-6, 07-239-13, and 07-239-15; and 07-240-2, 07-240-6, 07-240-13, and 07-240-15 were obtained from Chinese 166, Druchamp, Tyee, and Hyak based on their number positions in the 20 differential set for the two original isolates 07-239 and 07240, respectively. Urediniospores of each sub-isolate were used to test the whole set of wheat stripe rust differential genotypes as described above.

To further purify the isolates, single-uredium (or pustule) isolates were obtained from

the sub-isolates. Seedlings of Chinese 166, Druchamp, Tyee, and Hyak (two pots each; one for each sub-isolate) were inoculated with urediniospores from a sub-isolate. One leaf was inoculated with urediniospores from one uredium. After incubation in the dew chamber for 24 h and in the growth chamber for 14 days, urediniospores produced from each inoculated leaf were collected and designated as a single-uredium isolate. At least 3 single-uredium isolates were obtained from each of the sub-isolates. The single-uredium isolates were named as 07-238-2-Spu1, 07-238-2-Spu2, 07-238-2Spu3; 07-238-6-Spu1, 07-238-6-Spu2, 07238-6-Spu3, and so on. Urediniospores of these single-uredium isolates were increased on seedlings of corresponding differential genotypes, Chinese 166, Druchamp, Tyee, and Hyak to obtain enough spores for keeping in the stripe rust collection and further testing on the whole set of differential genotypes. After new virulence patterns were confirmed with the single-uredium isolates, new races were designated using selected single-uredium isolates as type isolates to represent the new virulence patterns based on the race designation system used in our program (Line and Qayoum 1992; Chen et al. 2002; Chen 2005).

RESULTS AND DISCUSSION

New race PST-127 was identified from isolates collected from IDO377s

Two isolates 07-239 and 07-240 were collected from IDO377s in the Whitlow Farm at Pullman, Washington in July 2007. Both isolates were virulent on wheat differentials Lemhi, Chinese 166, Heines, Paha, Drumchamp, Produra, Yamhill, Stephens, Lee, Fielder, Tyee, Hyak, Express, Yr8, Yr9, Clement, and Compair, but avirulent on Moro, Yr5, and Tres among the 20 differential genotypes. All of the sub-isolates and single-uredium isolates showed the same virulence pattern as the original isolates. These data indicated that the two isolates were genetically pure regarding their virulence or avirulence on the 20 differential

genotypes. The results of the virulence tests showed that they belonged to the same race (Table 1). The comparison of their virulence pattern with those of all previously identified races indicated that the two isolates belonged to a new race we designated as PST-127.

So far, a total of 137 races of *P. striiformis* f. sp. *tritici* have been identified in the United States (Chen 2005, 2007; Chen et al. unpublished data). In 2007, 19 previously identified races and 11 new races were identified from samples collected from USA (Table 2. Chen et al., unpublished data). Races with new virulences and new combinations of previously existing virulences that circumvent resistance genes in wheat cultivars are most important to disease management and wheat breeding programs. PST-127, PST-132, PST-135, PST-136, and PST- 137 with new virulences on differentials 13 (Tyee with resistance gene *YrTye*) and 15 (Hyak with *Yr17* and *YrTye*) should catch attention. Races PST-127 and PST-137 have new virulences on Tyee and Hyak plus virulences to *Yr8* and *Yr9* in most predominant races since 2000. PST-127 has virulences to all differentials except for Moro (*Yr10* and *YrMor*), *Yr5*, and Tres (*YrTr1* and *YrTr2*). This race also was detected from one sample collected from California in 2007. The appearance of the Hyak virulent races is a warning for use *Yr17* alone in breeding programs. As new races spread quickly in the recent years, these *Yr17* virulent races could spread from California and Washington to other states.

The new race PST-127 identified in this study should be noticed by the wheat breeders who are using IDO377s in their breeding pedigree. All tested 108 wheat breeding lines with or without IDO377s in the background were susceptible to the new race PST-127 but only 16.7% to the races PST-43 (virulences on Lemhi, Heines VII, Moro, Paha, Fielder, and Tres) and PST-45 (Lemhi, Heines VII, Fielder, Tyee, and Hyak). A total of 90 lines showed resistance to PST-43 and PST-45 were susceptible to PST-127. These data indicated that PST-127 could overcome *YrIDO377s*. Although PST-127 was avirulent on Moro and Tres, the resistance genes in these two cultivars have been circumvent by PST-114 and PST-116

appeared in 2004 and 2005, respectively (Table 2. Chen et al., unpublished data). According to the virulence pattern of PST-127, the *Yr5* gene is still resistant to this highly virulent race. *YrIDO377s* single gene lines developed in this study can be used as additional differential cultivars to monitor and identify new virulence pattern of *P. striiformis* f. sp. *tritici*.

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Table 1. Infection types produced by *Puccinia striiformis* f. sp. *tritici* isolates collected from IDO377s in 2007 on wheat differential genotypes

Isolate[a]	Infection types produced by *P. striiformis* f. sp. *tritici* isolates on wheat differentials[b]																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
07-239	8	8	8	2	8	8	2	8	8	8	8	8	8	2	8	8	8	8	8	8
07-240	8	8	8	2	8	8	2	8	8	8	8	8	8	2	8	8	8	8	8	8
07-239-2-spu1	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	7	8	8	8	8
07-239-2-spu2	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	8	8	8	8	8
07-239-2-spu3	8	8	8	2	8	8	2	7	8	8	8	8	8	2	8	8	8	8	8	8
07-239-6-spu1	8	8	8	2	8	8	2	7	8	8	8	8	8	2	8	8	8	8	8	6
07-239-6-spu2	8	8	8	2	8	8	2	8	8	8	8	8	8	2	8	7	8	8	8	8
07-239-6-spu3	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	8	8	8	8	8
07-239-13-spu1	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	8	8	8	8	8
07-239-13-spu2	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	7	8	8	8	8
07-239-13-spu3	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	6	8	8	8	6
07-239-15-spu1	8	8	8	2	8	8	2	8	8	8	8	8	8	2	8	8	8	8	8	8
07-239-15-spu2	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	8	8	8	8	8
07-239-15-spu3	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	6	8	8	8	8
07-240-2-spu1	8	8	8	2	8	8	2	8	8	8	8	8	8	2	8	8	8	8	8	8
07-240-2-spu2	8	8	8	2	8	8	2	7	8	8	8	8	8	2	8	8	8	8	8	8
07-240-2-spu3	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	8	8	8	8	8
07-240-6-spu1	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	8	8	8	8	8

07-240-6-spu2	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	8	8	8	8	8
07-240-6-spu3	8	8	8	2	8	8	2	7	8	8	8	8	8	2	8	6	8	8	8	8
07-240-13-spu1	8	8	8	2	8	8	2	6	8	8	8	8	8	2	8	8	8	8	8	8
07-240-13-spu2	8	8	8	2	8	8	2	7	8	8	8	8	8	2	8	8	8	8	8	8
07-240-13-spu3	8	8	8	2	8	8	2	8	8	8	8	8	8	2	8	7	8	8	8	8
07-240-15-spu1	8	8	8	2	8	8	2	7	8	8	8	8	8	2	8	8	8	8	8	8
07-240-15-spu2	8	8	8	2	8	8	2	7	8	8	8	8	8	2	8	8	8	8	8	8
07-240-15-spu3	8	8	8	2	8	8	2	8	8	8	8	8	8	2	8	8	8	8	8	8

[a] The isolate number was encoded by year-isolate number-differential number-single pustule number. e.g. 07-239-2-spu1 means single-uredium isolate 1 from sub-isolate 07-239-2 of the original isolate 239 collected in 2007 increased on wheat differential 2 (Chinese 166).

[b] Wheat differential genotypes 1-20 are: 1 = Lemhi, 2 = Chinese 166, 3 = Heines VII, 4 = Moro, 5 = Paha, 6 = Druchamp, 7 = Yr5, 8 = Produra, 9 = Yamhill, 10 = Stephens, 11 = Lee, 12 = Fielder, 13 = Tyee, 14 = Tres, 15 = Hyak, 16 = Express, 17 = Yr8, 18 = Yr9, 19 = Clement, and 20 = Compair.

TABLE 2. Races of *Puccinia striiformis* f. sp. *tritici* (PST) identified in 2007, virulence descriptions, race frequency and distribution.

PST race	Virulence formula[a]	1st year detected	No. of isolates	Frequency (%)	Distribution state (no.)
PST-21	2	1980	1	0.8	CA(1)
PST-25	1,3,6,8,9,10,12	1982	1	0.8	WA(1)
PST-37	1,3,6,8,9,10,11,12	1987	1	0.8	WA(1)
PST-40	1,4,14	1989	1	0.8	ID(1)
PST-45	1,3,12,13,15	1990	1	0.8	WA(1)
PST-80	1,3,8,11,12,16,17,18,19,20	2000	1	0.8	LA(1)
PST-97	1,3,10,11,12,16,17,18,19,20	2002	1	0.8	LA(1)
PST-98	1,3,8,10,11,12,16,17,18,19,20	2002	9	6.8	WA(5), AR(1), NE(1), KS(1), CA(1)
PST-100	1,3,8,9,10,11,12,16,17,18,19,20	2003	4	3.0	WA(2), NE (1), CO (1)
PST-101	1,2,3,8,9,10,11,12,16,17,18,19,20	2003	1	0.8	CA(1)
PST-102	1,3,8,9,10,11,12,14,16,17,18,19,20	2003	8	6.1	AR(1), CA(2), VA(1), WA(1), NE(1), KS(1), MT(1)
PST-108	1,3,4,6,9,10,12	2003	1	0.8	WA(1)

PST-111	1,3,5,8,10,11,12,16,17,18,19,20	2004	6	4.6	WA(5), MT(1)
PST-113	1,2,3,8,9,10,11,12,14,16,17,18,19,20	2004	7	5.3	CA(7)
PST-114	1,3,4,8,9,10,11,12,14,16,17,18,19,20	2004	22	16.7	WA(19), OR(2), ID(1)
PST-115	1,3,5,8,9,10,11,12,14,16,17,18,19,20	2004	10	7.6	WA(6), CA(1), ID(1), SD(1), KS(1)
PST-116	1,3,4,5,8,9,10,11,12,14,16,17,18,19,20	2005	21	15.9	WA(11), ID(7), OR(3)
PST-117	1,3,6,8,9,10,11,12,14,16,17,18,19,20	2005	6	4.6	WA(2), AR(1), KS(1), NE(1), ID(1)
PST-122	1,2,3,6,8,9,10,11,12,14,16,17,18,19,20	2006	4	3.0	CA(4)
PST-127	1,2,3,5,6,8,9,10,11,12,13,15,16,17,18,19,20	2007	2	1.5	WA(1), CA(1)
PST-128	1,2,3,6,8,10,11,12,16,17,18,19,20	2007	2	1.5	CA(2)
PST-129	1,2,3,8,10,11,12,16,17,18,19,20	2007	3	2.3	CA(3)
PST-130	1,3,4,8,10,11,12,16,17,18,19,20	2007	6	4.6	WA(3), OR(3)
PST-131	1,3,5,6,8,9,10,11,12,14,16,17,18,19,20	2007	3	2.3	WA(2), CO(1)
PST-132	1,3,6,8,9,10,12,13,15	2007	1	0.8	WA(1)
PST-133	1,2,3,5,6,8,9,10,11,12,14,16,17,18,19,20	2007	2	1.5	CA(2)
PST-134	1,3,6,9,10,11,12,14,16	2007	2	1.5	WA(2)
PST-135	1,3,5,6,8,9,10,12,13,15	2007	1	0.8	WA(1)

PST-136	1,3,9,10,12,13,15	2007	2	1.5	WA(1), CA(1)
PST-137	1,2,3,5,6,8,9,10,12,13,15,17,18,19,20	2007	1	0.8	CA(1)
	Total		132		

*1 = Lemhi, 2 = Chinese 166, 3 = Heines VII, 4 = Moro, 5 = Paha, 6 = Druchamp, 7 = Yr5, 8 = Produra, 9 = Yamhill, 10 = Stephens, 11 = Lee, 12 = Fielder, 13 = Tyee, 14 = Tres, 15 = Hyak, 16 = Express, 17 = Yr8, 18 = Yr9, 19 = Clement, and 20 = Compair.

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