Virulence and Molecular Characterization of Experimental Isolates of the Stripe Rust Pathogen (*Puccinia striiformis*) Indicate Somatic Recombination

Yu Lei, Meinan Wang, Anmin Wan, Chongjing Xia, Deven R. See, Min Zhang, and Xianming Chen

First and sixth authors: Agricultural College, Sichuan Agricultural University, Chengdu, Sichuan 611130, China; first, second, third, fourth, fifth, and seventh authors: Department of Plant Pathology, Washington State University, Pullman 99164-6430; and fifth and seventh: United States Department of Agriculture–Agricultural Research Service, Wheat Health, Genetics, and Quality Research Unit, Pullman, WA 99164-6430. Accepted for publication 14 October 2016.

ABSTRACT

Puccinia striiformis causes stripe rust on wheat, barley, and grasses. Natural population studies have indicated that somatic recombination plays a possible role in *P. striiformis* variation. To determine whether somatic recombination can occur, susceptible wheat or barley plants were inoculated with mixed urediniospores of paired isolates of *P. striiformis*. Progeny isolates were selected by passing through a series of inoculations of wheat or barley genotypes. Potential recombinant isolates were compared with the parental isolates on the set of 18 wheat or 12 barley genotypes that are used to differentiate races of *P. striiformis* f. sp. *tritici* (the wheat stripe rust pathogen) and *P. striiformis* f. sp. *hordei* (the barley stripe rust pathogen), respectively, for virulence changes. They were also tested with 51 simple-sequence repeat and 90 single-nucleotide polymorphism markers for genotype changes. From

68 possible recombinant isolates obtained from nine combinations of isolates based on virulence tests, 66 were proven to be recombinant isolates by molecular markers. Various types of recombinants were determined, including lost virulence from both virulent parental isolates, gained virulence from both avirulent isolates, combined virulences from both parents, and inherited virulence from one parent and avirulence from another. Marker data indicate that most of the recombinants were produced through chromosome reassortment and crossover after the hybridization of two parental isolates. The results demonstrate that somatic recombination is a mechanism by which new variants can be generated in *P. striiformis*.

Additional keywords: somatic hybridization, yellow rust.

Puccinia striiformis Westend. is an obligate biotrophic basidiomycete fungus causing stripe rust (yellow rust) on wheat, barley, and many grass species. Wheat stripe rust, caused by forma specialis *P. striiformis* f. sp. *tritici* Erikss., is one of the most destructive diseases of wheat in the world (Chen 2005; Stubbs 1985; Wellings 2011). The disease has been reported in over 60 countries (Chen 2005) and can cause severe yield losses of wheat worldwide, especially in China and the United States (Chen 2005; Chen et al. 2014; Wan et al. 2007). The most effective method to control stripe rust is to grow resistant cultivars (Chen 2005, 2013). However, virulent races evolve rapidly and can render previously resistant cultivars susceptible within only a few years (Chen 2005; Line and Qayoum 1992; Stubbs 1985; Wan et al. 2004; Wellings and McIntosh 1990). A large number of *P. striiformis* f. sp. *tritici* races have been identified in the United States, China, and other countries, and new races are continually identified every year (Chen 2005; Chen et al. 2002, 2010; Sharma-Poudyal et al. 2013; Wan and Chen 2012, 2014; Wan et al. 2007, 2016; Zhan et al. 2012).

It is important to understand the mechanisms of virulence and genetic variation in the *P. striiformis* population. Such information is crucial for deploying resistance genes in commercial cultivars for better control of stripe rust.

Genetic variability in *Puccinia* spp. has been reported to originate from mutation, sexual recombination, and somatic recombination (Knott 1989). The mechanisms by which new variants are created in *P. striiformis* were not fully understood (Steele et al. 2001). Somatic hybridization has been hypothesized as an evolutionary mechanism for the stripe rust fungus (Goddard 1976; Little and Manners 1969a,b; Taylor 1976; Wright and Lennard 1980). Somatic hybridization was thought to involve the fusion of dikaryotic hyphae followed by the reassociation of intact nuclei (heterokaryosis), or by nuclear fusion and segregation (parasexuality and somatic recombination) (Burdon and Silk 1997; Ellingboe 1961; Nelson et al. 1955). Heterokaryosis, which is a prerequisite for somatic hybridization and the parasexual cycle, was a possible mechanism of variation after obtaining strains with new virulence combinations from coinoculation of an albino isolate and eight Chinese races of *P. striiformis* f. sp. *tritici* (Kang et al. 1993, 1994a,b; Ma et al. 1993). Microscopic observations supported these results, because nuclear fusion in germ tubes was observed (Ma et al. 1993).

Early empirical studies attempting to demonstrate somatic recombination for *P. striiformis* focused on virulence attributes and isozyme phenotypes but largely did not suggest the presence of somatic recombination (Newton et al. 1986). It was difficult to detect and confirm naturally occurring somatic hybrids in the rust pathogen, due to the lack of isolate- or pathotype-specific markers (Park et al. 1999). A recent study of natural *P. striiformis* f. sp. *tritici* isolates suggests that genetic recombination has occurred on grasses between *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei*, the barley stripe rust pathogen (Cheng and Chen 2009; Cheng et al. 2016). However, it is not clear whether such genetic recombination has been through somatic recombination or sexual recombination because *P. striiformis* has been recently reported to

Corresponding author: X. Chen; E-mail address: xianming@wsu.edu

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be able to reproduce sexually on *Berberis* and *Mahonia* spp. under controlled conditions (Jin et al. 2010; Wang and Chen 2013). Although natural infection of *Berberis* plants at low frequencies has been detected in China (Wang et al. 2016; Zhao et al. 2013), no *P. striiformis* f. sp. *tritici* has been found on barberry plants in the United States (Wang et al. 2015). In fact, alternate hosts are ruled out from the *P. striiformis* f. sp. *tritici* reproduction in the U.S. Pacific Northwest by telial degradation after winter and the phenology of barberry plants (Wang and Chen 2015).

In 2009, a study was initiated in the senior author's lab to determine somatic recombination in *P. striiformis*; 84 isolates were obtained from coinoculation of wheat plants with mixed urediniopores

of nine combinations of isolates of *P. striiformis* (Cheng et al. 2015). These experimental isolates appeared to be different from their parental isolates based on tests with the previous set of 20 wheat cultivar differentials for identifying *P. striiformis* f. sp. *tritici* races and the set of 12 barley differentials (Chen 2005; Chen et al. 1995; Cheng et al. 2015; Wan and Chen 2012). However, due to the limitation of molecular markers for studying *P. striiformis*, only four isolates were found to be recombinants with only one or two simple-sequence repeat (SSR) markers (Cheng et al. 2015), and most potential recombinant isolates were not molecularly characterized. Moreover, the virulence differences of most isolates from the parental isolates could not be determined to be caused by mutation, somatic hybridization, or

TABLE 1. Infection types of parental and somatic recombinant isolates on 18 *Yr* single-gene lines of wheat used to differentiate races of *Puccinia striiformis* f. sp. *tritici*

Combination, isolate ^b	Infection type produced on wheat <i>Yr</i> single-gene lines ^a																		OC ^c	Race
	1	5	6	7	8	9	10	15	17	24	27	32	43	44	SP	Tr1	Exp2	Tye		
Combination 1 (A _w +C _o)																				
A _w (albino isolate)	1	1	7	5	1	1	1	1	2	2	2	2	1	1	1	2	3	1	100000	PSTv-19
C _o (PST-43)	1	1	8	3	1	1	8	1	8	8	5	8	1	1	1	8	1	1	105504	Unnamed
AC5 _o [(A+C)-1-10 _w -4 _o]	1	1	7	5	1	1	8	1	8	8	1	8	1	8	1	8	1	1	105524	New-1
AC7 _o [(A+C)-1-10 _w -14 _w -14 _o]	1	1	7	3	1	1	8	1	8	8	1	8	1	1	1	8	3	1	105504	Unnamed
AC8 _o [(A+C)-1-3 _o -1 _o -3 _o]	1	1	7	3	1	1	8	1	8	8	1	8	1	8	1	8	1	1	105524	New-1
AC10 _w [(A+C)-1-10 _w -12 _w -12 _w]	1	1	7	2	1	1	1	1	2	2	2	1	1	1	1	2	1	1	100000	PSTv-19
Combination 2 (A _w + D _o)																				
A _w (albino isolate)	1	1	7	5	1	1	1	1	2	2	2	2	1	1	1	2	3	1	100000	PSTv-19
D _o (2K-044)	1	1	8	8	8	8	1	1	8	3	8	2	8	8	1	8	8	1	171266	PSTv-37
AD1 _o [(A+D)-1-3 _w -3sp1 _o -3 _o]	1	1	8	8	8	8	2	1	8	3	5	3	8	8	1	8	8	1	171066	PSTv-35
AD2 _w [(A+D)-1-3 _w -3sp1 _o -3 _o -N _w]	1	1	7	2	1	1	1	1	2	2	2	2	2	2	1	2	2	1	100000	PSTv-19
AD3 _o [(A+D)-1-3 _w -3sp2 _o -3 _o]	1	1	8	8	8	8	2	1	8	3	5	3	8	8	1	8	8	1	171066	PSTv-35
AD4 _o [(A+D)-1-3 _w -3sp4 _w -3 _o]	8	1	8	8	8	8	2	1	8	3	5	3	8	8	1	8	8	1	571066	New-2
AD5 _o [(A+D)-1-3 _o -1 _o -3 _o]	8	1	8	8	5	8	2	1	8	3	5	3	8	8	1	8	8	1	551066	New-3
AD15 _w [(A+D)-1-3 _o -1 _o -3 _o -N _w -YrSP _w]	1	1	7	2	1	1	1	1	2	2	2	1	1	1	1	2	2	1	100000	PSTv-19
Combination 3 (A _w + F _o)																				
A _w (albino isolate)	1	1	7	5	1	1	1	1	2	2	2	2	1	1	1	2	3	1	100000	PSTv-19
F _o (08-220)	8	1	8	8	8	8	1	1	8	2	8	1	8	8	1	2	8	8	571263	PSTv-11
AF1 _o [(A+F)-5 _w -5SP1 _o -5 _o]	8	1	8	8	8	8	1	1	8	2	8	1	8	8	1	2	8	8	571263	PSTv-11
AF2 _o [(A+F)-5 _w -5SP2 _o -5 _o]	8	1	8	8	8	8	1	1	5	2	8	1	8	8	1	7	8	8	570267	PSTv-85
AF3 _o [(A+F)-5 _w -5SP3 _o -5 _o]	8	1	8	8	8	8	1	1	8	2	8	1	8	8	1	2	8	8	571263	PSTv-11
AF4 _o [(A+F)-5 _w -5SP4 _o -5 _o]	8	1	8	8	8	8	1	1	5	2	8	1	8	8	1	2	8	1	570262	New-4
AF5 _o [(A+F)-5 _w -5SP7 _o -5 _o]	8	1	8	8	8	8	1	1	8	2	8	1	8	8	1	2	8	8	571263	PSTv-11
AF6 _o [(A+F)-5 _w -5SP8 _o]	8	1	8	8	8	8	1	1	8	2	8	1	8	8	1	2	8	8	571263	PSTv-11
AF7 _o [(A+F)-5 _w -1 _o -5 _o]	8	1	8	8	8	8	7	1	8	7	8	8	8	8	1	7	8	8	575767	PSTv-49
AF8 _o [(A+F)-5 _o -1 _o -5 _o]	8	1	8	8	8	8	1	1	8	2	8	1	8	8	1	2	8	8	571263	PSTv-11
Combination 4 (C+D)																				
C (PST-43)	1	1	8	3	1	1	8	1	8	8	5	8	1	1	1	8	1	1	105504	Unnamed
D (2K-044)	1	1	8	8	8	8	1	1	8	3	8	2	8	8	1	8	8	1	171266	PSTv-37
CD1 [(C+D)-4-10]	1	1	8	3	1	1	8	1	8	8	5	8	5	7	1	8	5	1	105524	New-1
CD2 [(C+D)-5-2]	8	1	8	8	8	8	1	1	8	3	7	3	8	8	1	8	8	1	571266	PSTv-47
CD3 [(C+D)-5-8]	1	1	8	5	8	8	1	1	8	3	7	3	8	8	1	8	8	8	131267	New-5
CD4 [(C+D)-5-10]	1	1	8	5	8	8	1	1	8	8	2	8	8	2	1	8	1	8	135545	New-6
CD5 [(C+D)-5-12]	1	1	8	8	8	8	1	1	8	3	7	5	8	8	1	8	8	8	571367	New-7
CD6 [(C+D)-5-16]	1	1	8	8	8	8	1	1	8	3	7	3	8	8	1	8	8	1	171266	PSTv-37
CD8 [(C+D)-10-5]	1	1	8	8	8	8	1	1	8	3	5	1	1	8	1	8	8	8	171027	New-8
CD10 [(C+D)-12-14]	8	1	8	2	2	8	1	1	8	3	7	3	8	8	1	8	8	8	511267	New-9
CD11 [(C+D)-14-19]	1	1	8	8	8	8	1	1	8	3	7	3	8	8	1	8	8	1	171266	PSTv-37
CD12 [(C+D)-16-5]	1	1	8	8	8	8	1	1	8	3	7	3	8	8	1	8	8	1	171266	PSTv-37

(continued on next page)

^a The 18 wheat *Yr* single-gene lines are AvSYr1NIL (*Yr1*), AvSYr5NIL (*Yr5*), AvSYr6NIL (*Yr6*), AvSYr7NIL (*Yr7*), AvSYr8NIL (*Yr8*), AvSYr9NIL (*Yr9*), AvSYr10NIL (*Yr10*), AvSYr15NIL (*Yr15*), AvSYr17NIL (*Yr17*), AvSYr24NIL (*Yr24*), AvSYr27NIL (*Yr27*), AvSYr32NIL (*Yr32*), AvS/IDO377s F₃-41-1 (*Yr43*), AvS/Zak 1-1-35-line1 (*Yr44*), AvSYrSPNIL (*YrSP*), AvSYrTres1NIL (*YrTr1*), AvS/Exp 1/1-1 Line 74 (*YrExp2*), and Tyee (*YrTye*). Infection types 0 to 6 were considered avirulent and 7 to 9 virulent (Wan and Chen 2014). Infection types obviously different from either of the parental isolates are in bold.

^b All parental isolates have orange-colored uredinia, except the A_w isolate with white uredinia. If a combination has a white isolates and an orange isolate, the urediniospore color of the parental or a progeny isolate is indicated by "o" for orange color or "w" for white color. The isolate name within brackets [] indicates how the isolate was derived. For example, isolate AC5_o is an orange-urediniospored isolate developed from the infection by mixed urediniopores of parental isolate A (white-urediniospored) and isolate C (orange-urediniospored), and selected sequentially from urediniopores (mixed colors) produced on old wheat differential 1 (Lemhi, susceptible to both parental isolates), differential 10 (Stephens, susceptible to parental isolate A and resistant to isolate C) with mostly white urediniopores, and differential 4 (Moro, susceptible to parental isolate C and resistant to isolate A) with orange urediniopores. For combinations between a *P. striiformis* f. sp. *tritici* isolate and *P. striiformis* f. sp. *hordei* isolate, "D" denotes wheat differentials; "B" denotes barley differentials; "N" denotes Nugaines wheat (susceptible to all *P. striiformis* f. sp. *tritici* races and resistant to *P. striiformis* f. sp. *hordei* races; "Yr17", "Tr1", "Yr1", and "Yr9" denote wheat *Yr* single-gene lines with *Yr17*, *YrTr1*, *Yr1*, and *Yr9*, respectively; and "PI" denotes PI 478214 wheat (susceptible to all tested *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* isolates). See the caption of Figure 1 and refer to Wan and Chen (2012) for the numbers and names of the wheat and barley differentials.

^c Octal code (OC) is based on Wan and Chen (2014) and Wan et al. (2016).

somatic recombination. Since then, we have established a new set of *Yr* single-gene differentials for identifying *P. striiformis* f. sp. *tritici* races, which can be used to determine individual avirulences corresponding to specific resistance genes rather than to differential cultivars with two or more resistance genes (Wan and Chen 2014; Wan et al. 2016). In addition, more SSR markers and single-nucleotide polymorphism (SNP) markers have been developed for *P. striiformis* (Cheng et al. 2012; Tian et al. 2016; Xia et al. 2016a,b). The new single-gene line differentials allow us to better characterize virulences of *P. striiformis* populations, and the large number of codominant SSR and SNP markers are more suitable for studying recombinants of the dikaryotic fungus.

The objectives of the present study were to characterize the potential recombinant isolates of *P. striiformis* using the new set of

Yr single-gene line differentials and identify more somatic recombinants using a large number of SSR and SNP markers. This study should provide much stronger evidence for somatic recombination than the previous report (Cheng et al. 2015), and further identify different types of recombination. The results may improve our understanding of the *P. striiformis* evolution and lead to improved control of the stripe rust diseases on cereal crops.

MATERIALS AND METHODS

Pathogen materials. Seven *P. striiformis* f. sp. *tritici* isolates and two *P. striiformis* f. sp. *hordei* isolates from the United States were selected based on their virulence differences on wheat or

TABLE 1. (continued from preceding page)

Combination, isolate ^b	Infection type produced on wheat <i>Yr</i> single-gene lines ^a																		OC ^c	Race
	1	5	6	7	8	9	10	15	17	24	27	32	43	44	SP	Tr1	Exp2	Tye		
CD13 [(C+D)-14-10]	1	1	8	8	8	8	8	1	8	8	5	8	5	8	1	8	5	1	175524	New-10
CD15 [(C+D)-17-5]	1	1	8	5	8	8	1	1	8	3	7	3	8	8	1	8	8	1	131266	New-11
CD16 [(C+D)-18-14-14]	1	1	8	8	8	8	1	1	8	3	7	5	8	8	1	8	8	1	171266	PSTv-37
CD17 [(C+D)-20-5-5]	1	1	8	8	8	8	1	1	8	3	7	3	8	8	1	8	8	1	171266	PSTv-37
CD18 [(C+D)-18-5-5]	1	1	8	8	8	8	1	1	8	3	7	3	8	8	1	8	8	1	171266	PSTv-37
CD19 [(C+D)-14-20]	1	1	8	8	8	8	1	1	8	3	5	3	8	8	1	8	8	1	171066	PSTv-35
CD37 [(C+D)-12-5]	1	1	8	8	8	8	1	1	8	3	7	3	8	8	1	8	8	1	171266	PSTv-37
CD40 [(C+D)-1-1-2]	1	1	8	2	1	1	1	1	8	3	7	3	8	8	1	8	8	1	101266	New-12
CD42 [(C+D)-14-11]	1	1	8	8	8	8	1	1	8	3	7	3	8	8	1	8	1	1	171264	New-13
Combination 5 (C+F)																				
C (PST-43)	1	1	8	3	1	1	8	1	8	8	5	8	1	1	1	8	1	1	105504	Unnamed
F (08-220)	8	1	8	8	8	8	1	1	8	2	8	1	8	8	1	2	8	8	571263	PSTv-11
CF1 [(C+F)-14-10]	1	1	8	3	1	1	8	1	8	8	1	8	1	8	1	8	2	1	105524	New-14
CF2 [(C+F)-16-4]	1	1	8	8	8	1	8	1	8	8	8	8	8	8	1	2	8	8	165763	New-15
CF3 [(C+F)-12-6]	8	1	8	8	8	8	1	1	8	2	8	1	8	8	1	2	8	8	571263	PSTv-11
CF4 [(C+F)-14-6]	1	1	3	2	1	1	8	1	8	8	6	8	1	8	1	8	2	1	005524	New-16
CF5 [(C+F)-12-15]	8	1	8	8	8	8	1	1	8	2	8	1	8	8	1	2	8	8	571263	PSTv-11
Combination 6 (G+D)																				
G (07-147-13)	1	1	8	2	1	1	1	1	8	3	1	2	1	8	1	1	2	8	101021	Unnamed
D (2K-044)	1	1	8	8	8	8	1	1	8	3	8	2	8	8	1	8	8	1	171266	PSTv-37
GD1 [(G+D)-8-6]	1	1	8	8	8	8	1	1	8	3	8	3	8	8	1	8	8	1	171266	PSTv-37
GD2 [(G+D)-8-15]	1	1	8	8	8	8	1	1	8	3	5	3	8	8	1	8	8	8	171067	New-17
GD3 [(G+D)-8-13]	1	1	8	8	8	8	1	1	8	3	8	3	5	8	1	8	8	8	171227	New-18
GD4 [(G+D)-13-8]	1	1	8	3	1	1	1	1	8	3	3	5	3	8	1	1	2	8	101021	Unnamed
GD6 [(G+D)-15-8]	1	1	8	3	1	8	1	1	8	3	8	2	1	8	1	8	2	8	111215	New-19
GD7 [(G+D)-15-16]	1	1	8	8	8	8	1	1	8	3	8	3	8	8	1	8	8	8	171267	New-20
GD8 [(G+D)-15-17]	1	1	8	8	8	8	1	1	8	2	8	5	8	8	1	8	8	8	171267	New-20
GD9 [(G+D)-15-20]	1	1	8	8	8	8	1	1	8	3	2	2	5	8	1	8	8	8	171027	New-8
GD11 [(G+D)-20-6]	1	1	8	8	8	8	1	1	8	3	8	8	8	8	1	8	8	1	171366	New-21
GD12 [(G+D)-20-15]	1	1	8	8	8	8	1	1	8	3	8	3	8	8	1	8	8	1	171266	PSTv-37
Combination 7 (A _w +M _O)																				
A _w (albino isolate)	1	1	7	5	1	1	1	1	2	2	2	2	1	1	1	2	3	1	100000	PSTv-19
M _O (PSH-72)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	000000	PSTv-18
AM5 _O [(A+M)-D1 _w -B7 _O -B7 _O]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	000000	PSTv-18
AM6 _O [(A+M)-D10 _w -B1 _O -B1 _O]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	000000	PSTv-18
AM9 _O [(A+M)-D10 _w -B8 _O -B8 _O]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	000000	PSTv-18
AM10 _w [(A+M)-D10 _w -B8 _O -B8 _O -N _w]	1	1	7	2	1	1	1	1	2	2	2	2	2	2	1	2	2	1	100000	PSTv-19
AM15 _w [(A+M)-D10 _w -B1 _O -N _w -D10 _w -Yr17 _w -2]	1	1	7	2	1	1	1	1	2	2	2	2	2	2	1	2	2	1	100000	PSTv-19
AM19 _w [(A+M)-D10 _w -B8 _O -B8 _O -Tr1 _w]	1	1	7	2	1	1	1	1	2	2	2	2	1	2	1	2	2	1	100000	PSTv-19
AM25 _w [(A+M)-D10 _w -B1 _O -N _w -D10 _w -Yr1 _w -1]	1	1	7	2	1	1	1	1	2	2	1	2	2	2	1	2	2	1	100000	PSTv-19
AM26 _w [(A+M)-D1 _w -Yr17 _w -4]	1	1	7	2	1	1	1	1	2	2	2	2	2	2	1	2	2	1	100000	PSTv-19
AM28 _w [(A+M)-D10 _w -B1 _O -N _w -D10 _w -Yr9 _w -1]	1	1	7	5	1	1	1	1	2	1	2	2	1	2	1	2	2	1	100000	PSTv-19
Combination 8 (B+H)																				
B (PST-35)	1	1	7	5	1	1	1	1	5	4	5	5	1	1	1	2	4	1	100000	PSTv-19
H (07-176-S; PSH-33)	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	2	000000	PSTv-18
BH2 [(B+H)-PI-B1-D10]	1	1	7	5	1	1	1	1	5	4	5	5	1	2	1	2	4	1	100000	PSTv-19
BH4 [(B+H)-PI-D10-B1]	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	2	000000	PSTv-18
Combination 9 (E+H)																				
E (03-161-10-Sp1; PST-105)	7	1	8	7	7	1	8	1	7	7	5	7	8	8	1	7	8	2	565566	Unnamed
H (07-176-S; PSH-33)	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	2	000000	PSTv-18
EH1 [(E+H)-D1-B1]	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	2	000000	PSTv-18
EH2 [(E+H)-D1-B1]	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	2	000000	PSTv-18
EH3 [(E+H)-D8-B7]	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	2	000000	PSTv-18
EH4 [(E+H)-D10-B1]	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	2	000000	PSTv-18
EH5 [(E+H)-D17-B1]	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	2	000000	PSTv-18

barley genotypes used to differentiate races of the two formae speciales. The isolates were coded as A, B, C, D, E, F, and G for the seven *P. striiformis* f. sp. *tritici* isolates and H and M for the two *P. striiformis* f. sp. *hordei* isolates for convenience of description and discussion (Tables 1 and 2). Isolate A is white-urediniospored and has the same virulence formula as isolate B, both being classified as *P. striiformis* f. sp. *tritici* race PST-35 based on the previous set of 20 wheat cultivars differentials. Isolates B, C, D, E, F, and G have the typical orange urediniospores and different virulence patterns. Isolate C belongs to race PST-43, D to PST-98, E to PST-105, F to PST-127, and G to PST-135 based on reactions on the previous 20 wheat cultivar differentials for identifying *P. striiformis* f. sp. *tritici* races (Chen 2005; Chen et al. 2010; Wan and Chen 2012). The two *P. striiformis* f. sp. *hordei* isolates, H and M, are orange-urediniospored and belong to races PSH-33 and PSH-72, respectively (Wan and Chen 2012).

Plant materials. ‘Nugaines’ wheat, which was susceptible to all *P. striiformis* f. sp. *tritici* races at the seedling stage (Chen et al. 2002), and ‘Steptoe’ barley, which is susceptible to all *P. striiformis* f. sp. *hordei* races (Chen and Line 1999), were used to increase urediniospores of selected parental *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* isolates, respectively. ‘Lemhi’ wheat, which is susceptible to all *P. striiformis* f. sp. *tritici* races, except for race PST-21, identified thus far in the United States (Chen et al. 2002, 2010; Wan and Chen 2012), was used to increase urediniospores produced from inoculations of mixed urediniospores of *P. striiformis* f. sp. *tritici* isolate combinations. Wheat genotype ‘PI 478214’, which is susceptible to all tested *P. striiformis* f. sp. *tritici* and *P. striiformis*

f. sp. *hordei* races (Chen et al. 1995; Pahalawatta and Chen 2005), was used to increase urediniospores produced from inoculations of mixed urediniospores of *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* isolates. The 18 wheat *Yr* single-gene lines (Tables 1 and 2), which were recently established to differentiate races of *P. striiformis* f. sp. *tritici* (Wan and Chen 2014; Wan et al. 2016), were used to characterize virulence patterns of the potential recombinant isolates and their “parental” isolates of all of the combinations. For the combinations between a *P. striiformis* f. sp. *tritici* isolate and a *P. striiformis* f. sp. *hordei* isolate, the 12 barley varieties, which were used to differentiate *P. striiformis* f. sp. *hordei* races (Chen et al. 1995; Wan and Chen 2012), were also used to test the potential recombinants and their parental isolates.

Planting, inoculation, and differential testing. Urediniospores of each isolate taken from a liquid nitrogen tank were heat shocked at 50 to 55°C for 2 min. To increase urediniospores of each isolate, approximately 15 seeds of susceptible Nugaines wheat (or Steptoe barley) were planted in a plastic pot filled with a potting mixture obtained by mixing 24 liters of peat moss, 8 liters of perlite, 12 liters of sand, 12 liters of commercial potting soil, 16 liters of vermiculite, and 250 g of Osmocote (Chen and Line 1992). Seedlings were grown in a rust-free greenhouse (diurnal temperature cycle of 10 to 25°C) and inoculated uniformly with urediniospores of the isolate mixed with talcum (1:20) at the two-leaf stage (approximately 10 days after planting). Inoculated plants were placed in a dew chamber at 10°C in dark for 24 h, then incubated in a growth chamber in a greenhouse with diurnal temperatures gradually changing between 4°C at 2 A.M.

TABLE 2. Infection types of parental and somatic recombinant isolates on 12 cultivars of barley used to differentiate races of *Puccinia striiformis* f. sp. *hordei*

Combination, isolate ^b	Infection type produced on barley differentials ^a												Race ^c
	1	2	3	4	5	6	7	8	9	10	11	12	
Combination 7 (A _w +M _O)													
A _w (albino PST isolate)	2	1	1	1	1	1	2	1	1	1	1	1	NA
M _O (PSH-72)	8	7	8	7	7	8	8	8	8	8	8	8	PSH-72
AM5 _O [(A+M)-D1 _w -B7 _O -B7 _O]	8	2	2	2	2	2	8	8	2	2	2	8	PSH-54
AM6 _O [(A+M)-D10 _w -B1 _O -B1 _O]	8	7	8	8	7	8	8	8	8	8	8	8	PSH-72
AM9 _O [(A+M)-D10 _w -B8 _O -B8 _O]	8	2	2	2	1	2	8	2	1	2	2	2	PSH-33
AM10 _w [(A+M)-D10 _w -B8 _O -B8 _O -N _w]	2	1	1	1	1	1	1	1	1	1	1	1	NA
AM15 _w [(A+M)-D10 _w -B1 _O -N _w -D10 _w -Yr17 _w]	8	1	1	1	1	1	1	1	1	1	1	1	PSH-48
AM19 _w [(A+M)-D10 _w -B8 _O -B8 _O -Tr1 _w]	8	1	1	1	1	1	1	1	1	1	1	1	PSH-48
AM25 _w [(A+M)-D10 _w -B1 _O -N _w -D10 _w -Yr1 _w]	2	1	1	1	1	1	1	1	1	1	1	1	NA
AM26 _w [(A+M)-D1 _w -Yr17 _w]	2	1	1	1	1	1	1	1	1	1	1	1	NA
AM28 _w [(A+M)-D10 _w -B1 _O -N _w -D10 _w -Yr9 _w]	2	1	1	1	1	1	1	1	1	1	1	1	NA
Combination 8 (B+H)													
B (PST-35)	1	1	1	1	1	1	1	1	1	1	1	1	NA
H (07-176-S; PSH-33)	8	1	1	1	1	1	8	1	1	1	1	1	PSH-33
BH2 [(B+H)-PI-B1-D10]	1	1	1	1	1	1	1	1	1	1	1	1	NA
BH4 [(B+H)-PI-D10-B1]	8	1	1	1	1	1	8	1	1	1	1	1	PSH-33
Combination 9 (E+H)													
E (03-161-10-Sp1; PST-105)	1	1	1	1	1	1	1	1	1	1	1	1	NA
H (07-176-S; PSH-33)	8	1	1	1	1	1	8	1	1	1	1	1	PSH-33
EH1 [(E+H)-D1-B1]	8	1	1	1	1	1	8	1	1	1	1	1	PSH-33
EH2 [(E+H)-D1-B1]	8	1	1	1	1	1	8	1	1	1	1	1	PSH-33
EH3 [(E+H)-D8-B7]	8	1	1	1	1	1	8	1	1	1	1	1	PSH-33
EH4 [(E+H)-D10-B1]	8	1	1	1	1	1	8	1	1	1	1	1	PSH-33
EH5 [(E+H)-D17-B1]	8	1	1	1	1	1	8	1	1	1	1	1	PSH-33

^a The 12 barley differentials are 1 = Topper (susceptible to all races of *P. striiformis* f. sp. *hordei*), 2 = Heils Franken (*Rps4*, *rpsHF*), 3 = Emir (*rpsEm1*, *rpsEm2*), 4 = Astrix (*Rps4*, *rpsAst*), 5 = Hipoly (*rpsHi1*, *rpsHi2*), 6 = Varunda (*rpsVa1*, *rpsVa2*), 7 = Abed Binder 12 (*rps2*), 8 = Trumpf (*rpsTr1*, *rpsTr2*), 9 = Mazurka (*Rps1.c*), 10 = Bigo (*Rps1.b*), 11 = I 5 (*Rps3*, *rpsI5*), and 12 = Bancroft (*RpsBa*) (Chen et al. 1995; Chen and Line 2003; Wan and Chen 2012). Infection types 0 to 6 were considered avirulent and 7 to 9 virulent (Wan and Chen 2014).

^b All parental isolates have orange-colored uredinia, except the A_w isolate with white uredinia. If a combination has a white isolates and an orange isolate, the urediniospore color of the parental or a progeny isolate is indicated by “o” for orange color or “w” for white color. The isolate name within brackets [] indicates how the isolate was derived. For example, isolate AC5_O is an orange-urediniospored isolate developed from the infection by mixed urediniospores of parental isolate A (white-urediniospored) and isolate C (orange-urediniospored), and selected sequentially from urediniospores (mixed colors) produced on old wheat differential 1 (Lemhi, susceptible to both parental isolates), differential 10 (Stephens, susceptible to parental isolate A and resistant to isolate C) with mostly white urediniospores, and differential 4 (Moro, susceptible to parental isolate C and resistant to isolate A) with orange urediniospores. For combinations between a *P. striiformis* f. sp. *tritici* isolate and *P. striiformis* f. sp. *hordei* isolate, “D” denotes wheat differentials; “B” denotes barley differentials; “N” denotes Nugaines wheat (susceptible to all *P. striiformis* f. sp. *tritici* races and resistant to *P. striiformis* f. sp. *hordei* races; “Yr17”, “Tr1”, “Yr1”, and “Yr9” denote wheat *Yr* single-gene lines with *Yr17*, *YrTr1*, *Yr1*, and *Yr9*, respectively; and “PI” denotes PI 478214 wheat (susceptible to all tested *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* isolates). See the caption of Figure 1 and refer to Wan and Chen (2012) for the numbers and names of the wheat and barley differentials.

^c NA = not applicable.

and 20°C at 2 P.M., with a 16-h photoperiod (Chen and Line 1992). To prevent cross-contamination among isolates, plants were separated from each other in individual plastic booths. Approximately 15 days after inoculation, urediniospores were harvested with vacuum spore collectors and stored in a desiccator at 4°C for less than 2 months or at -80°C after being dried for a longer period (Chen and Line 1992; Chen et al. 2002).

A new set of 18 *Yr* single-gene differentials (Wan and Chen 2014; Wan et al. 2016) and 12 barley differentials (Chen and Line 2001; Chen et al. 1995) used to differentiate races of *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei*, respectively, were included to confirm the purity of each isolate and to determine races. Five wheat differential genotypes or four barley differential cultivars were spot planted in 7-by-7-by-7-cm plastic pots. Five to seven seeds of each differential cultivar were planted. Inoculation, incubation, and greenhouse conditions were as described previously (Chen and Line 1992; Wan and Chen 2014). Infection type (IT) on each wheat or barley genotype was recorded at 18 to 21 days after inoculation according to a 0-to-9 scale (Line and Qayoum 1992).

Experimental procedure. Nine combinations were established using the nine selected isolates (Tables 1 and 2). The combinations can be grouped into four types: (i) a white-spored isolate with an orange-urediniospored isolate of *P. striiformis* f. sp. *tritici* (A+C, A+D, and A+F); (ii) two orange-urediniospored isolates of *P. striiformis* f. sp. *tritici* (C+D, C+F, and G+D); (iii) a white-urediniospored isolate of *P. striiformis* f. sp. *tritici* and an orange-urediniospored isolate of *P. striiformis* f. sp. *hordei* (A+M); and (iv) an orange-urediniospored isolate of *P. striiformis* f. sp. *tritici* and an orange-urediniospored isolate of *P. striiformis* f. sp. *hordei* (B+H and E+H).

The schematic procedure for developing recombinant isolates is shown in Figure 1, using combination C+D as an example. The procedure can be divided into four steps. In step 1, approximately 90 seedlings of Lemhi (or PI 478214 for combinations involving a *P. striiformis* f. sp. *hordei* isolate) grown in six pots were inoculated at the two-leaf stage with the mixture of an equal quantity urediniospores of isolates C and D. Both isolates can infect and produce urediniospores on Lemhi. Approximately 2 weeks after inoculation, urediniospores produced on differential number 1 (Lemhi) (Chen et al. 2010) were collected with a vacuum spore collector, and the progeny isolate was marked isolate (C+D)-1. These urediniospores should be a mixture of parental isolates C and D and possible recombinants of C and D. In step 2, 60 seedlings of the wheat differentials 1 (Lemhi), 8 ('Produra'), 10 ('Stephens'), 11 ('Lee'), 16 ('Express'), 17 ('AvSYr8NIL'), 18 ('AvSYr9NIL'), 19 ('Clement'), and 20 ('Compair') (Chen et al. 2010) grown in four pots were inoculated with bulked urediniospores of isolate (C+D)-1. Urediniospores of isolate (C+D)-1-1 were harvested and kept in a desiccator at 4°C. Urediniospores were carefully harvested from differentials 8, 10, 11, 16, 17, 18, 19, and 20 and named (C+D)-1-8, -10, -11, -16, -17, -18, -19, and -20, respectively. Because isolate C could not infect or produce uredinia on these differentials, urediniospores collected from these differentials should be a mixture of parental isolate D and possible recombinants of C and D, without C. In step 3, 60 seedlings of the wheat differential cultivars 4 ('Moro'), 5 ('Paha'), and 14 ('Tres') grown in four pots were inoculated with bulked urediniospores of isolates (C+D)-1-8, -10, -11, -16, -17, -18, -19, and -20, and each leaf was carefully checked for the presence of uredinia on these differentials. Urediniospores from a single uredinium on these wheat differential cultivars were picked and inoculated on one leaf of fresh seedlings of the same differentials with a fine paintbrush. Because isolate D could not infect or produce uredinia on these three differentials, uredinia produced on these differentials should presumably arise through somatic recombination between parental isolates C and D. The presumed somatic recombinant (R) isolates were purified and multiplied by one or two additional cycles of inoculation and reisolation from single uredinia. These isolates were increased on the same differential cultivars until enough urediniospores were obtained for the later

virulence and molecular tests. These presumed R isolates were named with differential numbers or nondifferential variety abbreviations from which they were collected to keep the information of steps and originating differentials or varieties, and also with a number following the letters of the parental isolates (Tables 1 and 2) for easy description. In step 4, parental (C and D) and R isolates were tested for virulence patterns on the complete set of 20 wheat differentials, as described by Cheng et al. (2015). For combinations involving a *P. striiformis* f. sp. *tritici* isolate and a *P. striiformis* f. sp. *hordei* isolate, PI 478214 was used for the inoculation in step 1 because it is susceptible to all tested races of both formae speciales (Chen et al. 1995; Pahalawatta and Chen 2005). Inoculation, incubation, and greenhouse conditions were as described previously (Chen and Line 1992; Chen et al. 2002; Wan and Chen 2014).

Virulence analysis. Parental and presumed R isolates with virulence formulae distinct from their parental isolates on the set of 20 wheat differential cultivars (Cheng et al. 2015) were tested for virulence patterns on the new set of 18 *Yr* single-gene differentials (Wan and Chen 2014) and 12 barley differentials (Chen and Line 2001; Wan and Chen 2012) simultaneously. Differential tests for all presumed R isolates were conducted at least twice to confirm the different IT and virulence patterns. The previously described experimental procedures, conditions for growing plants before and after inoculation, methods of recording IT, converting IT to avirulence and virulence, and naming races were followed for identifying races using the new differentials and system (Wan and Chen 2014; Wan et al. 2016).

Molecular analysis. A modification of the cetyltrimethylammonium bromide method was used to extract DNA directly from urediniospores (Chen et al. 1993; Murray and Thompson 1980). DNA quality and concentrations were determined by electrophoresis (Maniatis et al. 1982) and spectrophotometry. DNA work solution at the concentration of 2 ng/μl, which was obtained for each isolate by diluting its stock solution with sterilized deionized H₂O, was used as template in polymerase chain reaction (PCR).

Fig. 1. Schematic experimental procedure for developing recombinant isolates on a set of 20 differential cultivars, using the combination (C+D), for example. Wheat differential cultivars: 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 = AvSYr 5NIL (*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 = AvSYr8NIL (*Yr8*), 18 = AvSYr9NIL (*Yr9*), 19 = Clement (*Yr9*, *YrCle*), and 20 = Compair (*Yr8*, *Yr19*) (Chen et al. 2010). W = white-urediniospored, O = orange-urediniospored, and R = presumed somatic recombinant isolates.

SSR markers. In total, 156 pairs of *P. striiformis* f. sp. *tritici* SSR primers (Bahri et al. 2009; Chen et al. 2009; Cheng et al. 2012; Enjalbert et al. 2002; Tian et al. 2016) were screened for useful markers, and the PCR conditions reported in these previous publications were followed. The primers of 51 SSR markers that were selected from the screening experiment were used to genotype the parental and presumed R isolates (Supplementary Table S1). PCR products were analyzed on an Applied Biosystems 3730xl DNA Analyzer (Thermo Fisher Scientific, Waltham, MA). The sizes of the PCR products were estimated using an internal DNA size standard, GeneScan 500 LIZ dye Size Standard (Thermo Fisher Scientific). Allele sizes in base pairs were called using software GeneMarker 4.0 (SoftGenetics LLC, State College, PA).

SNP markers. In total, 90 secreted protein gene-based SNP (SP-SNP) markers (Xia et al. 2016a,b) were screened with the parental isolates of *P. striiformis*. The PCR conditions and detection of SNP described by Xia et al. (2016a,b) were followed. The primers of 90 SP-SNP, together with their genomic locations based on supercontigs of the PST-78 genome sequence (https://www.broad-institute.org/annotation/genome/puccinia_group/MultiHome.html), are presented in Supplementary Table S2. Genotyping of the parental and R isolates at the SP-SNP loci was conducted on a Sequenom MassARRAY iPLEX platform (SEQUENOM, Inc., San Diego, CA).

Data analyses. Cluster analysis of the parental and presumed R isolates was conducted for each combination based on the virulence data and molecular data separately using software Past 3 (<http://folk.uio.no/ohammer/past/>). To determine the virulence relationships among the isolates, avirulent IT were converted to 0 and virulent IT to 1 following the methods of Chen et al. (1993) and Wan and Chen (2014). Dendrograms were constructed based on similarity coefficient using the unweighted pair group method with the arithmetic means. Bootstrap analysis was used to determine the relative robustness of branches of the dendrograms (Nelson et al. 1994), and branches with bootstrap values $\geq 50\%$ were considered highly robust. All of these analyses were performed using the Past 3 program (<http://folk.uio.no/ohammer/past/>). For each combination, two parental isolates were treated as different structures based on their SSR and SP-SNP markers, and the proportions of the markers from each parental isolate in progeny isolates were visualized using software DISTRUCT version 1.1 (Rosenberg 2004).

RESULTS

Virulence characterization. Of a total of 68 presumed R isolates used in this study, 59 were recovered from the previous study (Cheng et al. 2015) and 9 isolates were reisolated through inoculation of new differentials with previous isolates. Two white-urediniospored isolates, AD2_w and AM10_w, were reisolated from orange-urediniospored R isolates AD1_o and AM9_o, respectively; and seven white-urediniospored isolates (AC10_w, AD15_w, AM15_w, AM19_w, AM25_w, AM26_w, and AM28_w) were produced on new differentials inoculated with previous isolates. The IT of all 68 R isolates, together with their parental isolates, on the 18 *Yr* single-gene differentials are shown in Tables 1 and 2. Of the 68 R isolates, 15 (22.1%) had same virulence/avirulence patterns and IT as one of their parental isolates; 18 (26.5%) had a same virulence/avirulence pattern as one of their parental isolates but considerably different IT (e.g., 2 versus 5) on some differentials; and 35 (51.5%) had different virulence/avirulence patterns from either of their parental isolates. Of the 35 isolates with different patterns, 10 were identified as PSTv races that were previously detected from natural collections (Wan and Chen 2014; Wan et al. 2016), and 25 had virulence patterns different from all previously identified PSTv races based on the 18 *Yr* single-gene differentials.

The parental isolates A_w (white-urediniospored) and C_o (orange-urediniospored) in combination 1 differed in 5 virulences and shared 12 avirulences and 1 virulence on the 18 *Yr* single-gene differentials (Tables 1 and 2). Of the three orange-urediniospored

isolates, AC7_o had the same virulence pattern as the parental isolate C_o, and the other two (AC5_o and AC8_o) were identical in virulence patterns but had different IT on *Yr7*. A white-urediniospored isolate, AC10_w, had a virulence pattern similar to that of the parental isolate A_w but did not produce any uredinia on *Yr7* and *YrExp2*.

The parental isolates A_w and D_o (orange-urediniospored) in combination 2 differed in virulence on nine wheat differentials (Tables 1 and 2). We tested four orange-urediniospored and two white-urediniospored R isolates. The four orange-urediniospored isolates (AD1_o, AD3_o, AD4_o, and AD5_o) were similar to D_o (PSTv-37) but AD1_o and AD3_o were identified as PSTv-35 because they partially lost virulence to *Yr27*, and AD4_o and AD5_o were identified as new races because they lost avirulence to *Yr1*. Similarly, AD2_w and AD15_w had virulence patterns similar to that of parental isolate A_w but could not produce uredinia on *Yr7* and *YrExp2*. Interestingly, AD15_w [(A+D)-1-3_o-1_o-3_o-N_w-YrSP_w] was picked from the *YrSP* line. Considering the white color and lower aggressiveness of the white-urediniospored isolate, it was increased on PI 478214 to obtain enough urediniospores for further virulence tests and molecular characterization. When the isolate was tested on the differentials again, the virulence to *YrSP* was totally lost.

In combination 3 (A_w+F_o), parental isolate F_o (PSTv-11) was virulent on 11 of the 18 wheat differentials and isolate A_w was virulent on only 1 of them (Tables 1 and 2). Five R isolates (AF1_o, AF3_o, AF5_o, AF6_o, and AF8_o) had the same virulence pattern as F_o, whereas two isolates (AF2_o and AF7_o) were identified as different races (PSTv-85 and PSTv-49, respectively) and AF4_o as a new race.

The parental isolates of combination 4 (C+D) differed in reaction on 10 wheat differentials (Tables 1 and 2). Of the 19 R isolates, 10 had new virulence patterns different from each other and different from the parental isolates, 7 were identical to parental isolate D (PSTv-37), 1 (CD16) produced uredinia on the *Yr32* differential although it was still avirulent (IT 3), and 2 (CD2 and CD19) were identified as different races (PSTv-47 and PSTv-35, respectively). Although the parental isolates were both avirulent on *Yr1* and *YrTye*, six R isolates (CD2, CD3, CD4, CD5, CD8, and CD10) gained virulence to one or both genes, suggesting that both parental isolates were “heterozygous” at these avirulence loci.

The parental isolates of combination 5 (C+F) differed in reaction on 12 wheat differentials (Tables 1 and 2). Three R isolates had new and different virulence patterns, and two isolates were identified as PSTv-11, the same as parental isolate F. Isolate CF4 was avirulent on *Yr6*, whereas both parental isolates were virulent. The results suggested that the parental isolates were heterozygous at the virulence locus and that the virulence was dominant.

The parental isolates of combination 6 (G+D) differed in reaction on eight wheat differentials (Tables 1 and 2). Isolates GD1 and GD12 had the same virulence pattern as parental isolate D (PSTv-37). Each of the eight isolates had new and different virulence patterns.

Combinations 7 (A_w+M_o), 8 (B+H), and 9 (E+H) each were a mixture of a *P. striiformis* f. sp. *tritici* isolate and a *P. striiformis* f. sp. *hordei* isolate (Tables 1 and 2). Parental isolates in each of the combinations did not share any virulence on the wheat and barley differentials. The three orange-urediniospored R isolates of combination 7 (A_w+M_o) produced the same avirulence pattern on the wheat differentials as the *P. striiformis* f. sp. *hordei* parental isolate (M_o), and one white-urediniospored isolate (AM28_w) had almost the same IT as parent isolate A_w. The other five white-urediniospored isolates (AM10_w, AM15_w, AM19_w, AM25_w, and AM26_w) were similar to A_w but they lost the capability of producing uredinia on differentials of *Yr7* and *YrExp2*. Isolates AM15_w, AM19_w, AM25_w, AM26_w, and AM28_w were obtained from differentials of *Yr17*, *YrTr1*, *Yr1*, *Yr17*, and *Yr9*, respectively when testing previous isolates from this combination on the new set of differentials. As described for AD15_w, PI 478214 was used to increase the isolates. However, when enough white urediniospores were collected from PI 478214 and tested on the set of differentials again, their virulences on these differentials were completely lost.

In combination 8 (B+H), isolate BH2 had the same IT as the *P. striiformis* f. sp. *tritici* parental isolate B, whereas BH4 was identical to the *P. striiformis* f. sp. *hordei* parental isolate H on the wheat differentials. All five isolates derived from combination 9 (E+H) had the same reaction on the wheat differentials, which was the same as those of the *P. striiformis* f. sp. *hordei* parental isolate (H).

When tested on the barley differentials, the three orange-urediniospored R isolates from combination 7 (A_W+M_O) were found to be *P. striiformis* f. sp. *hordei* races different from the *P. striiformis* f. sp. *hordei* parental isolate M_O (orange-urediniospored) (Tables 1 and 2). Four ($AM10_W$, $AM25_W$, $AM26_W$, and $AM28_W$) of the six white-urediniospored R isolates had the same IT as the *P. striiformis* f. sp. *tritici* white-urediniospored parental isolate (A_W), whereas white-urediniospored isolates $AM15_W$ and $AM19_W$ gained virulence on barley differential 1 ('Topper'). The two R isolates BH2 and BH4 derived from combination 8 (B+H) produced the same reactions as B and H, respectively. In contrast, all R isolates derived from combination 9 (E+H) produced the same virulence pattern as the *P. striiformis* f. sp. *hordei* parental isolate (H) and, therefore, were identified as PSH-33.

In the virulence tests, all four possible types of recombination (recombined virulence, recombined virulence and avirulence, gaining partial virulence or full virulence [losing avirulence], and gaining avirulence [losing virulence]) were observed. An example of recombined virulence is shown in Figure 2A. Parental isolate G was avirulent on *YrExp2* and virulent on *YrTye* and parental isolate D was virulent on wheat differential *YrExp2* and avirulent on *YrTye*, whereas recombinant isolate GD2 had a combination of virulences

on both *YrExp2* and *YrTye*. An example of recombined virulence and avirulence is shown in Figure 2B. Parental isolate C was avirulent on wheat differential *Yr8* and *Yr9*, and the other parental isolate F was virulent on both, whereas recombinant isolate CF2 was virulent to *Yr8* and avirulence to *Yr9*. An example of gaining partial virulence (partially lost avirulence) is shown in Figure 2C. Parental isolate C was avirulent on wheat differential *YrExp2* and partially virulent on *Yr7* (IT 3), and parental isolate D was virulent on both. The R isolates CD1 and CD15 gained partial virulence on *YrExp2* (IT 4) and *Yr7* (IT 5). Both parental isolates C and D were avirulent to *YrTye*, whereas R isolate CD10 gained virulence to the gene. An example of gaining avirulence (losing virulence) is shown in Figure 2D. Parental isolate C was partially virulent and F was virulent on wheat differential *Yr27* but progeny isolate CF1 was avirulent to the gene. In addition, we also observed recombination between virulence and uredinial color. For example, the white-urediniospored parental *P. striiformis* f. sp. *tritici* isolate A_W was avirulent and the orange-urediniospored parental isolate C_O was partially virulent (IT5) to *Yr27*, whereas orange-urediniospored isolate $AC8_O$ was avirulent to the gene, the same as A_W (Fig. 2E). Gained virulence in a white-urediniospored isolate from the orange-urediniospored parental isolate is shown in Figure 2F. The parental *P. striiformis* f. sp. *tritici* isolate A_W (white-urediniospored) was avirulent (IT 3) and the parental *P. striiformis* f. sp. *hordei* isolate M_O (orange-urediniospored) was virulent (IT 8) on Topper, whereas the white-urediniospored R isolate $AM15_W$ was virulent (IT 7) on the barley variety.

Fig. 2. Infected leaves showing different recombination types compared with their parental isolates. **A**, Leaves of wheat differential lines with resistance gene *YrExp2* or *YrTye* inoculated with parental isolates G and D and progeny isolate GD2 of *Puccinia striiformis* f. sp. *tritici*. **B**, Leaves of wheat differential lines with resistance gene *Yr8* and *Yr9* inoculated with parental isolates C and F and progeny isolate CF2. **C**, Leaves of wheat differential lines with resistance genes *Yr7*, *YrExp2*, or *YrTye* inoculated with parental isolates C and D and progeny isolates CD1, CD10, and CD15. **D**, Leaves of wheat differential line with resistance gene *Yr27* inoculated with parental isolates C and D and progeny isolate CF1. **E**, Leaves of wheat differential lines with resistance gene *Yr6* or *Yr27* inoculated with parental isolate A_W (white-spored) of *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* isolate C_O (orange-spored) and progeny isolate $AC8_O$. **F**, Leaves of barley differential Topper and wheat differential *Yr6* line inoculated with parental *P. striiformis* f. sp. *tritici* isolate A (white-spored) and *P. striiformis* f. sp. *hordei* isolate M (orange-spored) and progeny isolates $AM25_W$ and $AM28_W$.

Molecular characterization. After screening with the parental isolates, 141 molecular markers (51 SSR and 90 SP-SNP) were used to genotype all parental and progeny isolates. Examples of SSR markers are shown in Figure 3 and SNP markers in Figure 4. The numbers of identical and polymorphic markers between the parental isolates in each combination and the number of markers identical and different from the parental isolates in each progeny isolate are shown in Table 3. The numbers of progeny isolates showing marker genotypes identical to or different from either of the parental isolates derived from each combination are presented in Table 4. Of the 68 progeny isolates from the nine combinations, 66 (97.1%) had at least one marker recombined from the parental alleles (Tables 3 and 4). Two major types of recombination were observed. The first major type was that an R isolate had a combination of alleles from both parental isolates. For example, parental isolate C had a homozygous allele of 191 bp and F had 194 bp, whereas the R isolate CF2 had the heterozygous alleles, combined from both parents (Fig. 3A). Another slightly different example from the first type is shown in Figure 3B. Both parental isolates C and D had the same heterozygous alleles of 263 and 266 bp but their progeny isolate CD2 had a homozygous 263-bp allele, one from each parent. The second major type was characterized by recombination between the urediniospore color and molecular markers, in which an R isolate derived from a combination of a white-urediniospored isolate and an orange-urediniospored isolates had the same molecular alleles at some of the marker loci as its parent of the opposite color. For example, of the potential R isolates derived from combination 7 (A_W+M_O), isolate AM28_W had a homozygous allele of 218 bp at the SSR SUNIPst09-48 locus, which was the same as the orange-urediniospored parental isolate M_O but different from the same-color parental isolate A_W (Fig. 3C, right). Similar to these types shown by SSR markers, these two major types were also observed with SNP markers (Fig. 4A to C).

It is interesting to observe similar but different alleles from those of either parent. For example, A_W had homozygous alleles of 128 bp and M_O had heterozygous alleles of 128 and 126 bp at the SSR CPS02 locus, whereas its progeny isolate AM25_W had two alleles: one 128-bp allele from either A_W or M_O and another 124-bp allele from neither of the parental isolates (Fig. 3C, left). Because the 124-bp allele is closer to the 126-bp allele, it might have arisen from an uneven crossover.

When the isolates derived from combinations A_W+C_O , A_W+D_O , and A_W+F_O and their parental isolates were tested using the 51 SSR primers, eight isolates (AC7_O, AF1_O, AF2_O, AF3_O, AF4_O, AF5_O, AF6_O, and AF7_O) did not have any marker to validate them as recombinants. However, these isolates had at least one SNP marker showing recombination.

In combination 4 (C+D), seven isolates (CD3, CD6, CD12, CD17, CD18, CD37, and CD42) had the same genotype as one of their parental isolates based on all 141 SSR and SNP markers. The remaining 12 isolates had marker patterns different from both parental isolates. Of the 12 isolates, CD15 had the combined alleles at 13 SSR markers and 4 SNP markers. These were all marker loci that were homozygous but different between the parental isolates C and D. The result indicated that CD15 was a hybrid with two intact nuclei, one from each of the parental isolates (Table 3).

Although all six isolates in combination 5 (C+F) had loci with combined alleles from each of their parental isolates C and F, the number of recombined loci ranged from 1 (CF1) to 46 (CF2). Unlike isolate CD15, CF2 did not have combined alleles from both parents at all 46 loci. Of the 15 SNP marker loci at which the two parental isolates were different and homozygous, 12 loci in CF2 had combined alleles from both parents and 3 loci (SNP-340, -422, and -431) were identical to C or F. Similarly, all progeny isolates derived from combination 6 (G+D) had at least one marker locus showing recombination (Table 3), except isolate GD4, which was identical in all SSR and SNP markers to parental isolate G.

Fig. 3. Simple sequence repeat (SSR) allele patterns of parental isolates and their progeny isolates. SSR banding patterns of parental isolates A, C and F and progeny isolate CF2 with primer scaffold443-47135; B, C and D and progeny isolate CD2 with primer C20508-14614; and C, A_W and M_O and progeny isolates AM25_W and AM28_W with primers CPS02 and SUNIPst09-48.

There were three orange-urediniospored and six white-urediniospored progeny isolates in combination 7 (A_W+M_O). The three orange-urediniospored isolates ($AM5_O$, $AM6_O$, and $AM9_O$) had either combined alleles from both parents or the same alleles as their white-urediniospored parent A_W in five, six, and three marker loci, respectively. Except for $AM19_W$, all white-urediniospored isolates had at least one locus of combined alleles from both parents or the same alleles as their orange-urediniospored parental isolate M_O . Isolate $AM26_W$ had combined alleles at three SSR and one SNP loci and had the same alleles as its orange-urediniospored parental isolate, M_O , at the SNP-47 locus. Interestingly, the isolate also had alleles from neither A_W nor M_O at five SSR and five SNP loci. For example, both parental isolates A_W and M_O had homozygous GG alleles, whereas $AM26_W$ had homozygous AA alleles at the SNP marker 431 locus. Similarly, A_W had a homozygous 129-bp allele and M_O had heterozygous 127- and 129-bp alleles at the SSR marker CPS02 locus, whereas $AM26_W$ had heterozygous 125- and 129-bp alleles at the locus. Isolate $AM25_W$ had a similar marker pattern.

Although many SSR and SNP markers can differentiate *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* isolates, only isolate BH2 had combined alleles from both parents at the SSR C13690-2968 locus among the progeny isolates in combination 8 (B+H) (Tables 3 and 4). However, molecular markers separated all five progeny isolates from their two parents and from each other, except for EH3 and EH5 in combination 9 (E+H).

Identification of crossover events by molecular markers. The supercontigs of the 141 SSR and SNP markers are presented in Supplementary Tables S1 and S2. Despite the fact that most markers were located in different supercontigs, markers in single supercontigs

were identified and used to identify possible crossover events. For example, SSR markers SUNIPst 11-21, SUNIPst 15-30, and PSP 62 were in supercontig 15 and SNP markers SNP-53, -64, -413, and -422 were in supercontig 11. In an example shown in Figure 5, parental isolate C had the allele (CG)(CC)(AG)(AA) and F had (CG)(CT)(AA)(GG), whereas progeny isolate CF2 had (CG)(CC)(AG)(GG) at the SNP loci of SNP-53, -64, -413, and -422, respectively. Although there are several possible explanations, including double crossover, the simplest explanation is a single crossover in the region between markers SNP-413 and SNP-422 in the chromosome of supercontig 11 of parental isolates C and F.

Structure analysis of R isolates. The number and percentage of SSR and SNP markers identical to and different from each parent and those combined from both parents for each progeny isolate were presented in Table 3. To visualize the proportions of markers (representing the proportions of the genomes) from each parental isolate in each progeny isolates, structure analysis was conducted, and the results are presented in Supplementary Figure S1. The results showed that most progeny isolates resulted from recombination, and the proportions of marker alleles from the two parental isolates in each R isolate was not equal, indicating uneven recombination of genomes of the parental isolates.

Comparison of virulence phenotypes and molecular genotypes. When the progeny isolates were analyzed separately using the virulence phenotype data and the marker genotype data, 36 (52.9%) isolates were shown to be races different from either of the parental isolates, and 9 (13.2%) isolates had considerable IT changes although they were typed as the same race as one of their parental isolates; thus, 45 isolates (66.2%) were considered as recombinants (Table 4). The molecular data showed a much higher number of

Fig. 4. Single-nucleotide polymorphism (SNP) patterns of parental isolates and progeny isolates. SNP banding patterns of parental isolates A, C and D and progeny isolate CD15 with primer SNP-398; B, C and F and progeny isolate CF2 with primer SNP-4; and C, A_W and M_O and progeny isolate $AM28_W$ with primers SNP-222 and SNP-309.

isolates, 66 (97.1%), as recombinant isolates. When the phenotypic and genotypic data were combined, only 1 isolate (AF5_O) still did not show any change, and 67 (98.5%) could be considered as recombinant isolates. These results can be more easily visualized by the clusters of isolates by phenotypic data and genotypic data (Fig. 6A to I). For example, the four progeny isolates and the two parental isolates in combination A_W+C_O were clustered into three clusters by the phenotype data but separated into six genotypes, showing that the four progeny isolates were different from each other and also different from either of the parental isolates (Fig. 6A). Similar results were observed for the isolates in combinations A_W+D_O (Fig. 6B), C+F (Fig. 6E), G+D (Fig. 6F), and B+H (Fig. 6H). Limited by the small number of differentials, some isolates could not be separated by the virulence phenotypic data but they could be separated by the marker genotypic data. This can be seen in all

combinations, and is most obvious in combination E+H, in which all five progeny isolates were identical to parental isolate H but clearly separated by marker data, except for isolates EH3 and EH5 (Fig. 6I). Occasionally, isolates had an identical genotype but differed in virulence phenotype, as we observed in CD37 and CD42 (Fig. 6D) and in A_W and A_W19 (Fig. 6G). Nevertheless, molecular markers, as a larger number of them were used, were more powerful in identification of recombinant isolates.

DISCUSSION

Previous studies obtained one or two races that could be considered to be produced from somatic recombination between isolates of *P. striiformis* f. sp. *tritici* (Goddard 1976; Little and Manners 1969a,b; Wright and Lennard 1980), and attempts to obtain

TABLE 3. Numbers of identical and polymorphic markers between the parental isolates, number of markers identical and different from the parental isolates in progeny isolates, and potential mechanisms

		Number of loci with alleles (%) ^a				
		Clearly from			Different ^d	Mech ^e
Cmb, isolate ^b	Loci (%) ^c	Paren 1	Paren 2	Both		
Cmb 1 (A _W +C _O)	70 (49.6)	...	...	...	...	...
A _W	...	...	...	...	...	...
C _O	...	...	...	...	...	...
AC5 _O	...	4 (5.7)	64 (91.4)	2 (2.9)	0 (0)	R
AC7 _O	...	3 (4.3)	66 (94.3)	1 (1.4)	0 (0)	R
AC8 _O	...	3 (4.3)	65 (92.9)	2 (2.9)	0 (0)	R
AC10 _W	...	65 (92.9)	3 (4.3)	2 (2.9)	0 (0)	R
Cmb 2 (A _W + D _O)	112 (79.4)	...	...	...	...	...
A _W	...	...	...	...	...	...
D _O	...	...	...	...	...	...
AD1 _O	...	4 (3.6)	106 (94.7)	2 (1.8)	0 (0)	R
AD2 _W	...	110 (98.2)	1 (0.9)	1 (0.9)	0 (0)	R
AD3 _O	...	7 (6.3)	102 (91.1)	3 (2.7)	0 (0)	R
AD4 _O	...	5 (4.5)	107 (91.1)	5 (4.5)	0 (0)	R
AD5 _O	...	4 (3.6)	104 (92.9)	4 (3.6)	0 (0)	R
AD15 _W	...	108 (96.4)	3 (2.7)	1 (0.9)	0 (0)	R
Cmb 3 (A _W + F _O)	106 (75.2)	...	...	...	...	...
A _W	...	...	...	...	...	...
F _O	...	...	...	...	...	...
AF1 _O	...	1 (0.9)	105 (99.1)	0 (0)	0 (0)	R
AF2 _O	...	1 (0.9)	105 (99.1)	0 (0)	0 (0)	R
AF3 _O	...	2 (1.9)	104 (98.1)	0 (0)	0 (0)	R
AF4 _O	...	2 (1.9)	103 (97.2)	1 (0.9)	0 (0)	R
AF5 _O	...	0 (0)	106 (100)	0 (0)	0 (0)	R
AF6 _O	...	1 (0.9)	105 (99.1)	0 (0)	0 (0)	R
AF7 _O	...	2 (1.9)	103 (97.2)	1 (0.9)	0 (0)	R
AF8 _O	...	2 (1.9)	102 (96.2)	2 (1.9)	0 (0)	R, C
Cmb 4 (C+D)	112 (79.4)	...	...	...	...	...
C	...	...	...	...	...	...
D	...	...	...	...	...	...
CD1	...	100 (89.2)	9 (8.0)	3 (2.7)	0 (0)	R
CD2	...	8 (7.1)	98 (87.5)	6 (5.4)	0 (0)	R
CD3	...	5 (4.5)	107 (95.5)	0 (0)	0 (0)	R
CD4	...	96 (85.7)	11 (9.8)	5 (4.5)	0 (0)	R, C
CD5	...	13 (11.6)	89 (79.5)	10 (8.9)	0 (0)	R
CD6	...	8 (7.1)	104 (92.9)	0 (0)	0 (0)	R
CD8	...	101 (90.2)	11 (9.8)	0 (0)	0 (0)	R
CD10	...	18 (16.1)	93 (83.0)	11 (9.8)	0 (0)	R, C
CD11	...	6 (5.4)	105 (93.8)	1 (0.9)	0 (0)	R
CD12	...	3 (2.7)	109 (97.3)	0 (0)	0 (0)	R
CD13	...	97 (86.6)	14 (12.5)	1 (0.9)	0 (0)	R, C

(continued on next page)

^a Paren = parental isolate.

^b Cmb = combination.

^c Number (and percentage) of polymorphic marker loci between parental isolates.

^d Different from either of the parental isolates.

^e Possible mechanism: R = reassortment, H = hybridization, C = crossover, and M = mutation.

^f AM 25w had one other allele at single-nucleotide polymorphism (SNP)-440 which was not included in the number of polymorphic marker loci between parental isolates.

^g AM26w had three other alleles at SNP-17, -431, and -66 which were not included in the number of polymorphic marker loci between parental isolates.

recombinants between *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* isolates failed (Newton et al. 1985). These early studies were limited by virulence, morphology (spore color), and molecular marker (isozyme) traits. Detection of genetic recombination in natural *P. striiformis* populations (Cheng and Chen 2009; Cheng et al. 2016; Mboup et al. 2009) was only suggestive of somatic recombination because sexual recombination is possible in some regions (Wang et al. 2016; Zhao et al. 2013). A large number of potential recombinant isolates was obtained under controlled experimental conditions and preliminary tests with a limited number of SSR markers suggested somatic recombination (Cheng et al. 2015). In the present study, we further characterized 59 available isolates from the previous study by Cheng et al. (2015) and 9 new isolates with the new

set of 18 Yr single-gene lines and 141 SSR and SNP markers. We were able to show 67 of the 68 progeny isolates to be recombinants based on virulence phenotypes or marker genotypes. The success in obtaining and demonstrating the large number of somatic recombinant isolates was achieved by choosing isolate combinations with a relatively large number of virulence differences, several combinations, and especially a large number of molecular markers. Although the possibility of contamination might not be completely ruled out, we were confident in the results of the R isolates because we took every measure to prevent possible contaminations. The parental isolates were used as controls in every step of the isolation and in the tests of virulence, SSR, and SNP markers. They remained the same in virulence and urediniospore color, and all marker bands were from the parental isolates. Furthermore, our

TABLE 3. (continued from preceding page)

Cmb, isolate ^b	Loci (%) ^c	Number of loci with alleles (%) ^a				Mech ^e
		Clearly from			Different ^d	
		Paren 1	Paren 2	Both		
CD15	...	20 (18.1)	73 (66.4)	17 (15.5)	0 (0)	H
CD16	...	3 (2.7)	105 (93.8)	4 (3.6)	0 (0)	R
CD17	...	3 (2.7)	109 (97.3)	0 (0)	0 (0)	R
CD18	...	7 (6.3)	105 (93.8)	0 (0)	0 (0)	R
CD19	...	1 (0.9)	108 (96.4)	3 (2.7)	0 (0)	R
CD37	...	2 (1.8)	109 (97.3)	1 (0.9)	0 (0)	R
CD40	...	101 (90.2)	9 (8.0)	2 (1.8)	0 (0)	R, C
CD42	...	2 (1.8)	109 (97.3)	1 (0.9)	0 (0)	R
Cmb 5 (C+F)	104 (73.8)	...	...	...	...	...
C	...	...	...	...	...	...
F	...	...	...	...	...	...
CF1	...	103 (99.0)	0 (0)	1 (1.0)	0 (0)	R
CF2	...	55 (52.9)	18 (17.3)	28 (26.9)	0 (0)	R, C
CF3	...	5 (4.8)	96 (92.3)	3 (2.9)	0 (0)	R
CF4	...	101 (97.1)	2 (1.9)	1 (1.0)	0 (0)	R
CF5	...	4 (3.8)	97 (93.3)	3 (2.9)	0 (0)	R
Cmb 6 (G+D)	111 (78.7)	...	...	...	...	...
G	...	...	...	...	...	...
D	...	...	...	...	...	...
GD1	...	9 (8.1)	95 (86.4)	6 (5.5)	0 (0)	R
GD2	...	91 (82.0)	12 (10.8)	8 (7.2)	0 (0)	R
GD3	...	102 (91.9)	7 (6.3)	2 (1.8)	0 (0)	R
GD4	...	98 (88.3)	13 (11.7)	0 (0)	0 (0)	R
GD6	...	104 (93.7)	6 (5.4)	1 (0.9)	0 (0)	R
GD7	...	5 (4.5)	103 (92.8)	3 (2.7)	0 (0)	R, C
GD8	...	9 (8.1)	97 (87.4)	5 (4.5)	0 (0)	R, C
GD9	...	4 (3.6)	103 (92.8)	4 (3.6)	0 (0)	R
GD11	...	7 (6.3)	102 (91.9)	2 (1.8)	0 (0)	R
GD12	...	6 (5.4)	104 (93.7)	1 (0.9)	0 (0)	R
Cmb 7 (A _w + M _O)	84 (59.6)	...	...	...	...	...
A _w	...	...	...	...	...	...
M _O	...	...	...	...	...	...
AM5 _O	...	2 (2.4)	79 (94.0)	3 (3.6)	0 (0)	R
AM6 _O	...	1 (1.2)	78 (92.9)	5 (6.0)	0 (0)	R
AM9 _O	...	1 (1.2)	80 (95.2)	3 (3.6)	0 (0)	R
AM10 _w	...	78 (92.9)	5 (6.0)	1 (1.2)	0 (0)	R, C
AM15 _w	...	82 (97.6)	2 (2.4)	0 (0)	0 (0)	R
AM19 _w	...	84 (100)	0 (0)	0 (0)	0 (0)	C
AM25 _w	...	80 (95.2)	2 (2.4)	1 (1.2)	1 (1.2)+1 ^f	R, C, M
AM26 _w	...	73 (86.9)	4 (4.8)	1 (1.2)	6 (7.1)+3 ^g	H, R, M
AM28 _w	...	75 (89.3)	7 (8.3)	2 (2.4)	0 (0)	R, C
Cmb 8 (B+H)	93 (66.0)	...	...	...	...	...
B	...	...	...	...	...	...
H	...	...	...	...	...	...
BH2	...	90 (96.8)	2 (2.2)	1 (1.1)	0 (0)	R
BH4	...	1 (1.1)	92 (98.9)	0 (0)	0 (0)	H, R
Cmb 9 (E+H)	83 (58.9)	...	...	...	...	...
E	...	...	...	...	...	...
H	...	...	...	...	...	...
EH1	...	1 (1.2)	82 (98.8)	0 (0)	0 (0)	R, C
EH2	...	1 (1.2)	82 (98.8)	0 (0)	0 (0)	R
EH3	...	1 (1.2)	82 (98.8)	0 (0)	0 (0)	R
EH4	...	1 (1.2)	82 (98.8)	0 (0)	0 (0)	R
EH5	...	1 (1.2)	82 (98.8)	0 (0)	0 (0)	R

cluster analysis clearly showed that every progeny isolate was closely related to one of its parental isolates, except two isolates (AM19_w and AF5_o) that were identical to one of their parents. Therefore, most of progeny isolates were clear recombinants.

Our virulence results of the R isolates on 18 *Yr* single-gene lines largely support the previous results that were based on 20 wheat

cultivar differentials (Cheng et al. 2015), although 15 progeny isolates did not show differences from one of their parental isolates when tested on the new set of differentials. These were due to the different differentials used in the two studies. In fact, we proved most of these isolates to be recombinants using SSR and SNP markers. When we used the combination of the virulence and marker data, 67 of 68 progeny isolates were validated as recombinants. The results clearly indicate that somatic recombination can occur, and possibly at a much higher frequency than previously thought, although this study did not attempt to determine recombination frequency.

Different types of recombination were identified, including loss of virulence from virulent or partial virulent parental isolates, gained of virulence from avirulent parental isolates, combined virulences to different resistance genes from both parents, and inherited virulence from one parent and avirulence from another. For virulence absent in both parental isolates but present in an R isolate, we can assume that both parental isolates are heterozygous (heterokaryotic) for that avirulence locus. Through nuclei reassortment, two nuclei, one from each parent, carrying the recessive virulence allele are inherited by a urediniospore to form a new isolate different from either of the parental isolates. In this case, virulence is recessive whereas avirulence is dominant. For an R isolate that did not get the virulence that is present in both parental isolates, we also can assume that both parents are heterozygous but, in this case, virulence is dominant. Such

Fig. 5. Crossover process between chromosomes of parental isolates C and F and their progeny isolate CF2 on supercontig 11 (Sc11) as an example.

TABLE 4. Progeny isolates showing virulence phenotypes or marker genotypes identical to one of the parental isolates or different from either of the parental isolates of *Puccinia striiformis*

Combination	N ^d	Virulence phenotype				Marker genotype	
		Number (isolates) as same race ^a		Number (isolates) as different race ^b		Number (isolates) ^c	
		Identical IT	Different IT	Named race	New race	Identical	Different ^e
1: A _w +C _o	4	0	2 (AC7 _o , AC10 _w)	0	2 (AC5 _o , AC8 _o)	0	4 [AC5 _o (6), AC7 _o (4), AC8 _o (5), AC10 _w (5)]
2: A _w +D _o	6	0	2 (AD2 _w , AD15 _w)	2 (AD1 _o , AD3 _o)	2 (AD4 _o , AD5 _o)	0	6 [AD1 _o (6), AD2 _w (2), AD3 _o (10), AD4 _o (10), AD5 _o (8), AD15 _w (4)]
3: A _w +F _o	8	5 (AF1 _o , AF3 _o , AF5 _o , AF6 _o , AF8 _o)	0	2 (AF2 _o , AF7 _o)	1 (AF4 _o)	1 [AF5 _o]	7 [AF1 _o (1), AF2 _o (1), AF3 _o (2), AF4 _o (3), AF6 _o (1), AF7 _o (3), AF8 _o (4)]
4: C+D	19	0	7 (CD6, CD11, CD12, CD16, CD17, CD18, CD37)	2 (CD2, CD19)	10 (CD1, CD3, CD4, CD5, CD8, CD10, CD13, CD15, CD40, CD42)	0	19 [CD1 (12), CD2 (14), CD3 (5), CD4 (16), CD5 (23), CD6 (8), CD8 (11), CD10 (29), CD11 (7), CD12 (3), CD13 (15), CD15 (37), CD16 (7), CD17 (3), CD18 (7), CD19 (4), CD37 (3), CD40 (11), CD42 (3)]
5: C+F	5	2 (CF3, CF5)	0	0	3 (CF1, CF2, CF4)	0	5 [CF1 (1), CF2 (38), CF3 (8), CF4 (3), CF5 (7)]
6: G+D	10	0	3 (GD1, GD4, GD12)	0	7 (GD2, GD3, GD6, GD15, GD16, GD21, GD22)	0	10 [GD1 (15), GD2 (20), GD3 (9), GD4 (13), GD6 (7), GD7 (8), GD8 (14), GD9 (8), GD11 (9), GD12 (7)]
7: A _w +M _o	9	1 (AM6 _o)	4 (AM10 _w , AM25 _w , AM26 _w , AM28 _w)	4 (AM5 _o , AM9 _o , AM15 _w , AM19 _w)	0	1 [AM19 _w]	8 [AM5 _o (5), AM6 _o (6), AM9 _o (4), AM10 _w (6), AM15 _w (2), AM25 _w (5), AM26 _w (14), AM28 _w (9)]
8: B+H	2	2 (BH2, BH4)	0	0	0	0	2 [BH2 (3), BH4 (1)]
9: E+H	5	5 (EH1, EH2, EH3, EH4, EH5)	0	0	0	0	5 [EH1 (1), EH2 (1), EH3 (1), EH4 (1), EH5 (1)]
Total (%)	68	15 (22.1)	18 (26.5)	10 (14.7)	25 (36.8)	2 (2.9)	66 (97.1)

^a Progeny isolates had identical or different infection types (IT) but races identical to one of their parental isolates with the same color of uredinia. Virulence data were based on the tests on the 18 *Yr* single-gene differentials of wheat; IT 0 to 6 are considered as avirulent and 7 to 9 as virulent (Wan and Chen 2014, Wan et al. 2016). If the two parental isolates in a combination have different-colored uredinia, “o” indicates orange uredinia and “w” indicates white uredinia.

^b Progeny isolates identified as a race different from both parental isolates, either as a named races previously identified from the natural population or not identified (new) race (Wan and Chen 2014, Wan et al. 2016).

^c Progeny isolates had genotypes identical to one of the parental isolates or different from either of the parental genotypes based on simple-sequence repeat (SSR) and single-nucleotide polymorphism (SNP) markers.

^d Number of progeny isolates.

^e Numbers in parentheses are the number of SSR or SNP markers different from one of the parental isolates with the most similar genotype.

findings and interpretation agree with the previous observation and interpretation by Little and Manners (1969a,b), Chen et al. (2012), and Tian et al. (2016). Based on this discussion, the somatic recombination approach may be used to determine inheritance of individual virulences or avirulences. This approach should be relatively easy compared with the sexual recombination approach.

In a previous study, Cheng et al. (2015) obtained 84 potential recombinant isolates based on virulence tests with the old set of U.S. differentials. Limited by the available markers, they found only 6 of 11 screened SSR markers polymorphic between some of the parental isolates. By testing 28 potential recombinant isolates using the six polymorphic SSR markers, they found only 4 isolates to be recombinants, of which 2 isolates had only one marker and 2 isolates had two markers. Their work provided preliminary results for supporting somatic recombination and a large number of experimental isolates for further characterization. However, their results could not indicate whether these isolates were produced by somatic hybridization or somatic recombination. The different alleles of the isolates at only one marker locus could not rule out the possibility of mutation. Furthermore, most potential recombinant isolates remained molecularly uncharacterized. In order to obtain stronger evidence for somatic recombination, from 156 SSR and 98 SNP markers, we selected 51 SSR and 90 SNP markers to characterize the 9 parental and 68 progeny isolates. Although all of the markers (except SNP-43, -56, -364, -407, -14, -45, and -181) were polymorphic between two parental isolates in at least one of the nine combinations, we tested the isolates with all of the markers. The use of a large number of monomorphic markers for each combination allowed us to ensure that the progeny isolates were not derived from contamination (data not shown). More importantly, the relatively large number of markers allowed us to obtain multiple markers for identification of recombinant isolates, which further allowed us to distinguish recombination from hybridization and identify crossover events in the development of some isolates.

In the testing of previous isolates on the *Yr* single-gene line differentials, we found that some white-urediniospored isolates produced white uredinia on *Yr1*, *Yr9*, *Yr17*, *YrTr1*, and *YrSP*. We reisolated samples from these wheat plants and increased on PI 478214. However, when we tested the subisolates on the differentials again, they were no longer able to produce uredinia on these *Yr* gene lines. The loss of capability to produce uredinia or virulence might be related to possible instability of heterokaryosis, as reported in *P. graminis* f. *tritici* (Nelson et al. 1955) and *P. tritici* (Guo et al. 1988). In fact, losing urediniospore productivity has been occasionally observed in natural *P. striiformis* f. sp. *tritici* samples when tested on some wheat differential varieties (X. M. Chen, unpublished data). Further virulence tests and molecular data demonstrated that these white-urediniospored isolates were not the same as the original albino isolate and demonstrated them as recombinants. The white color of uredinia greatly helped us to develop and identify recombinant isolates in the combinations involving the albino isolate and a normal-color isolate. It is worth noting that the white-urediniospored isolates AM25_w and AM26_w had alleles at some marker loci that did not come from either of their parental isolates. This might have been caused by mutation or uneven crossover, and should not be caused by contamination because only one white-urediniospored parental isolate was available. Further characterization of these isolates by sequencing may identify the genetic events.

The failure of a previous study attempting to observe somatic recombination between *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* led Newton et al. (1985) to postulate that hybridization between formae speciales of *P. striiformis* may not occur because of a genetic barrier. However, we obtained 16 R isolates in the three different combinations, each involving one *P. striiformis* f. sp. *tritici* isolate and one *P. striiformis* f. sp. *hordei* isolate, based on virulence characterization. We also proved 15 of them (except AM19_w) as recombinants by at least one marker. Our results do not support the

hypothesis of Newton et al. (1985) but do show that recombination between two formae speciales is possible. The results are similar to the findings in *P. graminis* (the stem rust pathogen) for which hybridization between formae speciales has been achieved experimentally (Luig and Watson 1972). The results of the present study may also support the speculation that some isolates collected from grasses are hybrids between field isolates of *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* (Cheng and Chen 2009; Cheng et al. 2016). The present study shows that somatic recombination can occur not only between isolates of the wheat stripe rust pathogen but also between isolates of the wheat and barley stripe rust pathogens. However, based on the number of R isolates, it appears that R isolates may be produced more easily between isolates of the same forma specialis than between different formae speciales.

Our marker data indicated that almost all of the progeny isolates were produced through somatic recombination, including cell fusion, nuclear reassortment (somatic hybridization), chromosomal reassortment, and crossover, and these events can occur during a complete process of parasexual cycle found in many fungi (Alexopoulos and Mims 1979). However, one isolate (CD15) was found to be formed through somatic hybridization without the further events.

Possible crossover events were identified in several recombinant isolates by organizing markers based on their supercontigs. Because of the dikaryotic nature of the pathogen, it was not possible in this study to determine the two haplotypes in each isolate. Therefore, for a recombinant isolate, there are different pairs of haplotypes, and also more than one possible genetic mechanism. Further studies are needed to determine structural changes in the genomes of recombinant isolates caused by crossover using a high-quality genome sequencing approach.

It was interesting to find that a recombinant isolate that was derived from a combination of a white-urediniospored isolate and an orange-urediniospored had the same “homozygous” molecular alleles at some of the marker loci as its parent of the opposite color. The best example of this type of recombinants was AM28_w, which had white uredinia like parental isolate A_w but had homozygous alleles of parental isolate M_O at several marker loci. Of the total 141 markers tested, 84 markers were polymorphic between the two parent isolates (Table 3). AM28_w had 75 marker loci at the homozygous state like A_w, 7 marker loci at the homozygous state like M_O, and only 2 marker loci at the heterozygous state (data not shown). These results suggested that the recombinant isolate was mostly similar to that of its same-color parental isolate, with less than 10% of the genome inserted from its different-color parental isolate. The other five white-urediniospored recombinant isolates derived from the same combination had similar marker genotypes, whereas the three orange-urediniospored recombinant isolates had more markers of the orange-urediniospored parental isolates and few markers of the white-urediniospored parental isolate. The color trait or color-trait-related chromosomes might play a role in the formation of the recombinant isolates.

Except in a few cases, most recombinant isolates had more markers identical to one of the parental isolates and fewer markers identical to another. Thus, the uneven genome contributions by the parental isolates appeared to be more common than relatively even contributions. These results suggested that, when a fusion occurred between cells of the two parental isolates after urediniospore germination or during the infection process within the leaf tissue, more than two nuclei might come together and exchange their chromosomes. Indeed, most infectious hyphal cells of *P. striiformis* f. sp. *tritici* in the host leaf tissue produced by urediniospore infection have more than two nuclei (Kang et al. 1994a). Crossovers may occur more often between higher homologous chromosomes than lower homologous chromosomes. Vegetative compatibility, mating types, or some mechanism resembling the clamp connection in smut fungi may prevent most chromosomes or chromosomal regions from the same original cell from entering into a new cell to form a recombined urediniospore. Recombinant urediniopores with

Continued on next page

Fig. 6. Dendrograms of parental and progeny isolates of nine combinations constructed based on the virulence phenotypic data and molecular genotype data. **A**, Combination 1 (A_W+C_O); **B**, combination 2 (A_W+D_O); **C**, combination 3 (A_W+F_O); **D**, combination 4 ($C+D$); **E**, combination 5 ($C+F$); **F**, combination 6 ($G+D$); **G**, combination 7 (A_W+M_O); **H**, combination 8 ($B+H$); and **I**, combination 9 ($E+H$).

fewer “foreign” chromosomes or genomic DNA may more likely survive than those with more foreign DNA. Thus, more isolates with uneven contributions from two different isolates can be obtained. This phenomenon may be a general rule for somatic recombination of *P. striiformis*, which may make it distinct from sexual recombination of a relatively more even contribution of genetic material from both parents. However, which mechanisms or factors determine uneven contributions observed in the present study requires further studies.

This study is the first to use a large number of DNA-based markers to characterize somatic recombination in *P. striiformis* isolates under controlled greenhouse conditions. The isolates can be used for further characterizing genome structural changes using

new sequencing technologies that can produce long sequences, which may result in more information on proportions of parental genomes in recombinant isolates and genome regions for recombination, as well as provide answers for the questions discussed above. The present study suggests that somatic recombination is a mechanism for the evolution of the stripe rust pathogen, and opens a new avenue to study the population biology and genetics of a pathogen by developing a large population, without the need to produce sexual crosses for characterization using a genome-wide associate study approach. The determination of dominant and recessive virulences can be useful for predicting new races and selecting effective resistance genes for use in developing resistant cultivars to control the disease.

Fig. 6. Continued from previous page.

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