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FULL RESEARCH PAPER

Relationships and genetics of wheat effects on infection frequency and colony extension of *Puccinia striiformis* f. sp. *tritici*

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Abstract Wheat cvs Aquileja and Xian Nong 4, previously reported to possess quantitative resistance to *Puccinia striiformis* f. sp. *tritici* (*Pst*), were crossed to assess resistance associated with infection frequency and colony extension of *Pst*. The parents, F_1, F_2, F_3, F_4, backcrossed to Aquileja, and backcrossed to Xian Nong 4 were sown in the field. Experimental plants were inoculated with a single-spore derived isolate of *Pst*. Penetration frequency, colony size and disease severity were quantified. Comparison between the parents indicated that Aquileja was better at limiting infection frequency of *Pst*, whereas Xian Nong 4 was better at restricting colony extension. Penetration frequency and colony size were controlled by different genes. It was estimated that three or four genes, and three genes, controlling penetration frequency and colony size respectively, segregated in the cross Aquileja × Xian Nong 4, and were transmitted with moderately high heritability in additive fashion with no detectable dominant and epistatic effects. The two traits were correlated with disease severity. Colony size and penetration frequency explained, respectively, up to 33 and 8% of the genotypic variation of disease severity. These results will be helpful in understanding quantitative resistance and breeding for enhanced resistance to *Pst*.

Keywords Components of quantitative resistance · Durable resistance · Gene action · Genetic correlation · Partial resistance · Stripe (yellow) rust · *Triticum aestivum*

Abbreviations

AQ	Aquileja
BC	backcross
MX	Ming Xian 169
Pst	*Puccinia striiformis* f. sp. *tritici*
XN	Xian Nong 4

Introduction

Yellow (stripe) rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most damaging diseases of wheat (*Triticum aestivum*) in many parts of the world (Stubbs 1988). Use of host resistance is a major way to control the disease. Resistance of the

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hypersensitive response conferred by single genes with major effects has been extensively used; this form of resistance is usually race-specific and thus can be overcome by new virulent *Pst* races (Johnson 1984; Stubbs 1988; Line 2002). Another form of resistance is slow-rusting, adult plant resistance, or high-temperature, adult-plant resistance involving genes of minor or partial effect (quantitative resistance). Although it is not a generality that quantitative resistance is durable, this form of resistance has proven to remain effective for a prolonged period of time in several cultivars, including Cappelle Desprez and Hybride de Bersée (Johnson 1984), Nugaines and Luke (Milus and Line 1986; Chen and Line 1995), *Yr18*-involved cultivars (Singh 1992), Aquileja and Xian Nong 4 (Zhang 1995; Zhang et al. 2001), and Camp Rémy (Mallard et al. 2005).

Quantitative resistance is the product of an interaction between host and pathogen at different stages of pathogenesis. A single-cycle infection by *Pst* can be divided into different stages of spore germination, penetration of stomata and formation of substomatal vesicles, colony extension, and sporulation (Mares and Cousen 1977; Vallavieille-Pope et al. 1995), and wheat cultivars may vary in response to *Pst* at these stages (Mares and Cousen 1977; Broers and López-Atilano 1996). While studies on wheat quantitative resistance to *Pst* have been well documented for the stages after appearance of visible symptoms (e.g. Milus and Line 1986; Chen and Line 1995; Zhang 1995; Zhang et al. 2001; Mallard et al. 2005), reports are limited for pre-symptom stages. Mares and Cousen (1977) showed that wheat resistance to *Pst* was associated with a reduced fungal colony growth rate. Broers and López-Atilano (1996) indicated that wheat cultivars differed in their ability to limit appressorium formation of *Pst*.

Information about the relationships and genetics of host effects on pathogen growth at pre-symptom stages of pathogenesis will be valuable for bettering our understanding of quantitative resistance. Enhanced resistance can be achieved by bringing together host effects expressed at different stages in one plant genotype.

The wheat cvs Aquileja and Xian Nong 4 have previously been reported to possess quantitative resistance to *Pst*, and transgressive segregation for disease severity in both directions of low value and high value was observed in the cross Aquileja×Xian Nong 4 (Zhang et al. 2001). In a separate study, the two cultivars were compared for their effects on *Pst* at different stages of pathogenesis (Feng et al. 2007). No substantial difference between the cultivars was detected at pre-penetration stages, and no difference in disease severity was observed at post-symptom stages. Aquileja was better at limiting *Pst* penetration than Xian Nong 4, whereas Xian Nong 4 was better at restricting *Pst* colony extension under the leaf epidermis.

The objectives of this study were to (a) determine the relationships between penetration frequency, colony size, and disease severity of *Pst* in the cross Aquileja×Xian Nong 4, (b) determine the nature of gene action (additive, dominant, and/or epistatic) in wheat for penetration frequency and colony size, (c) estimate the heritability and number of genes for penetration frequency and colony extension, and (d) examine if the transgressiveness for disease severity, as previously reported, is related to the combination of the genes conferring penetration frequency and those involved in colony extension.

Materials and methods

Host materials

The wheat (*T. aestivum*) cvs Aquileja (AQ) and Xian Nong 4 (XN) were used as parents in crossing, and the cv. Ming Xian 169 (MX) was used as a susceptible control. MX is highly susceptible to all known *Pst* races detected in China (Wan et al. 2004). AQ has the accession number PI393993 in the US Department of Agriculture GRIN database (Germplasm Resources Information Network (http://www.ars-grin.gov) with the pedigree of Tevere/ Giuliani// Gallini. XN and MX were deposited at the Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, Beijing 100081, China, with the accession numbers ZM023551 and ZM009379 (http://icgr.caas.net.cn), respectively. Both XN and MX are local Chinese cultivars with unknown pedigree. AQ and XN were crossed reciprocally, and the seeds of F_1, F_2, F_3 and F_4 were produced successively during the period 2001 to 2005. In addition, F_1 plants were crossed to AQ and XN, respectively, with F_1 as females, resulting in the backcross generations referred to as BC_{AQ} and BC_{XN}. These host materials

were evaluated for *Pst* resistance in the field in Beijing where the wheat-growing season lasts from early October to the following late June.

Pathogen materials and inoculation

It is unusual for *Pst* to occur naturally in Beijing (no such occurrence was observed during 2001–2006 when this study was conducted). Chinese race CYR32 of *Pst* was used for artificial inoculation which is virulent to resistance genes *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr22*, *Yr23*, *Yr27*, *YrA*, *YrCV1*, *YrCV2*, *YrCV3*, *YrG*, *YrSD*, and *YrSO* (Wan et al. 2004), and thus is similar in virulence to race 239E175 in the world and European race nomenclature (Johnson et al. 1972). From CYR32 (deposited at the Plant Protection Institute, Chinese Academy of Agricultural Sciences, Beijing 100094, China) single-spore isolates were derived and confirmed for their virulence identity. One of the isolates was used as inoculum. Inoculation was conducted in middle–late May when flag leaves were completely unfolded (39–45 on the Zadoks scale; Zadoks et al. 1974). Fresh uredospores suspended in water containing 0.02% Tween 20 were uniformly atomized on to all of the plants in the experiments, at an average density of 100 germinated spores per square centimeter leaf area, in the evenings when the temperature was 8–13°C. The inoculated plants were then covered with plastic film overnight to facilitate dew-formation and thus infection, and on the following morning the plastic film was removed when the temperature under the film was 16–19°C.

Field and microscopic quantification of pathogen growth in host plants

Seeds were sown in rows 2 m long at 8 cm intervals for F_3 and F_4 and at 15 cm intervals for the other generations with a 30-cm space between adjacent rows. The experiments were in a randomized complete-block design with three replications. AQ, XN and MX were sown every 15 test rows for convenient reference.

During the 2003–2004 season, 14–18 plants for each parent and F_1, and 295 plants for F_2 were used for measuring all the traits of penetration frequency, colony size and disease severity, and an additional 53–644 plants depending on generation were also evaluated for disease severity. From the 295 F_2 plants, 70 were randomly sampled to produce F_3 families. During the 2004–2005 season, seven generations were sown including AQ, XN, F_1, F_2, F_3, BC_{AQ} and BC_{XN}. The number of plants used for measuring all the traits of penetration frequency, colony size and disease severity are listed in Table 1, and 67–341 plants, depending on generation, were also rated for disease severity. From each of the 70 F_3 families, five plants were sampled to produce F_4 families. During the 2005–2006 season, 18–52 plants for each parent and F_1, and 70 F_4 family groups with five families in each group and five plants in each family were scored

Table 1 Penetration frequency (PF) and colony size (CS) of *Puccinia striiformis* f.sp. *tritici* in plants from the cross between wheat cvs Aquileja (AQ) and Xian Nong 4 (XN), together with the susceptible reference wheat cv. Ming Xian 169 (MX) tested in 2005

Trait[a]		Generation							
		AQ (18)[b]	XN (26)	F_1 (20)	F_2 (153)	BC_{AQ} (84)	BC_{XN} (84)	F_3 (700,70)[c]	MX (10)
PF	Mean±SD[d]	2.15±0.51	5.37±1.27	3.95±1.26	4.55±2.30	3.14±1.65	4.29±1.95	4.86±2.17	9.02±0.37
Log_{10}(CS)	Mean±SD	5.85±0.09	4.77±0.17	5.21±0.21	4.94±0.37	5.25±0.30	4.85±0.28	5.27±0.34	6.61±0.06
% control of disease[e]		9.5	15.3	12.9	13.4	12.3	13.1	14.1	100.0

[a] PF was determined as number of substomatal vesicle per square centimeter of leaf area. CS was measured in μm^2 of individual colony, and was log_{10}-transformed to improve normality.

[b] Figures in parenthesis are the numbers of plants observed in the experiment for respective generations.

[c] 70 F_3 families with ten plants from each family were observed.

[d] *SD* Standard deviation.

[e] Percent disease severity in each generation, compared to the susceptible control wheat cv. Ming Xian 169.

for disease severity. Eighteen to 20 days after inoculation when plants were at 55–65 on the Zadoks scale (Zadoks et al. 1974), disease severity, determined as percent of leaf area with *Pst* symptoms, was recorded on flag leaves of three tillers of each plant using the scale developed by Peterson et al. (1948).

A flag leaf segment of about 5 cm long from the middle part of the leaf was collected five days after inoculation from each of experimental plants except those of F_4. The leaf segments were cleared in a nearly-saturated solution of chloral hydrate (2.5 g dissolved in 1 ml of water). Fungal structures were stained by boiling the leaf segments for 10 min in alcoholic lactophenol trypan blue (20 ml ethanol, 10 ml phenol, 10 ml water, 10 ml lactic acid (83%), and 25 mg trypan blue). The leaf segments were then mounted under coverslips in 50% glycerol for microscopic observation (Frye and Innes 1998). The substomatal vesicles in a leaf segment were counted. The length and width of a leaf segment was measured, and thus the square centimeter area. Number of substomatal vesicles per square centimeter leaf area (SSV cm^{-2}) was taken as an estimate of penetration frequency for each plant. The maximum colony dimensions parallel to and at right angles to the leaf veins were measured in micrometers (μm) and thus an estimate of colony size in μm^2.

Statistical analyses

Because the segregating individuals did not separate into discrete classes, quantitative rather than Mendelian methods were used for analyzing the data. Frequency distribution was tested for normality by using the Kolmogorov–Smirnov D statistic at the $P_{>D} = 0.05$ level and the Shapiro–Wilk W statistic at the $P_{<W} = 0.05$ level (SAS Institute 1991).

Phenotypic correlation coefficients between penetration frequency, colony size, and disease severity were calculated on the data of F_2, F_3, BC_{AQ} and BC_{XN}. Genetic correlation coefficients were calculated for F_2/F_3 and F_3/F_4 data by using the equation of Falconer (1981): $r = COV_{XY}/\sqrt{(COV_{XX}COV_{YY})}$ where COV_{XY} is the 'cross-covariance' obtained from the product of the value of X trait in parents and the value of Y trait in offspring, and COV_{XX} and COV_{YY} are the offspring–parent covariances of each trait separately. The calculation was conducted using Proc Corr Pearson of SAS (SAS Institute 1991).

Genetic effects were estimated using joint scaling tests (Mather and Jinks 1982). Both models of additive-dominance and models containing digenic interactions (epistasis) were also tested. Additive-dominance models estimated the mid-parent value m, the additive genetic component [d], and the dominant component [h] without considering epistasis. In epistatic models, in addition to m, [d] and [h], one or more digenic interactions can be included: additive×additive interaction [i], additive×dominant interaction [j], and dominant×dominant interaction [l]. Eleven models were tested for fitting: m[d], m[h], m[d][h], m[d][i], m[h][l], m[d][h][i], m[d][h][j], m[d][h][l], m[d][h][i][j], m[d][h][i][l], and m[d][h][j][l]. Comparison between the means observed (Table 1) and the means expected on the basis of the component estimates was done by assuming the sum of squares minimized in the fitting process to be distributed as a chi-square (χ^2)·χ^2 test was conducted to determine how well a model fits the data with the criterion P=0.05, and simultaneously, the Student's t-test was conducted to determine if a genetic component in the model is different from zero at the P=0.05 level. A model would be accepted if all components are significantly different from zero by the t-test as well as if it fits the data by the χ^2 test.

Heritability was estimated by using two methods. Method 1: $h^2 = [2V_{F2} - (V_{BCP1} + V_{BCP2})]/V_{F2}$, in which V_{F2}, V_{BCP1} and V_{BCP2}=variances of F_2, BC_{P1} and BC_{P2} respectively, (Nyquist 1991). Method 2: $h_2 = COV(F_3/F_2)/\sqrt{(V_{F2}V_{F3})}$, in which $COV(F_3/F_2)$=covariance of F_3 and F_2, and V_{F2} and V_{F3}=the variances of F_2 and F_3, respectively. This is the regression of F_3 on F_2 on data coded in terms of standard deviation units (Frey and Horner 1957) that were identical to correlation coefficients in the original data. Standard errors of correlations were calculated according to Falconer (1981).

Number of minimum effective factors (called genes below for convenience) was estimated by using three methods. Method 1: $m_p = \left\{[(F_{2H} - F_{2L})h^2]^2 - (V_{PH} + V_{PL})/n\right\} \Big/ (8V_s)$, in which m_p=number of genes, F_{2H}−F_{2L}=phenotypic range between the F_2 plant with highest value and the plant with lowest value, h^2=heritability, V_{PH} and V_{PL}=variances of the parent with higher value and the parent with lower value respectively, n=total number of plants of P_H and P_L, and $V_s = 0.2(4V_{F2} + V_{BH} + V_{BL}) - 0.4(V_{PH} + V_{PL} + V_{F1})$ in which V_{F2}, V_{BH}, V_{BL}, and V_{F1}=

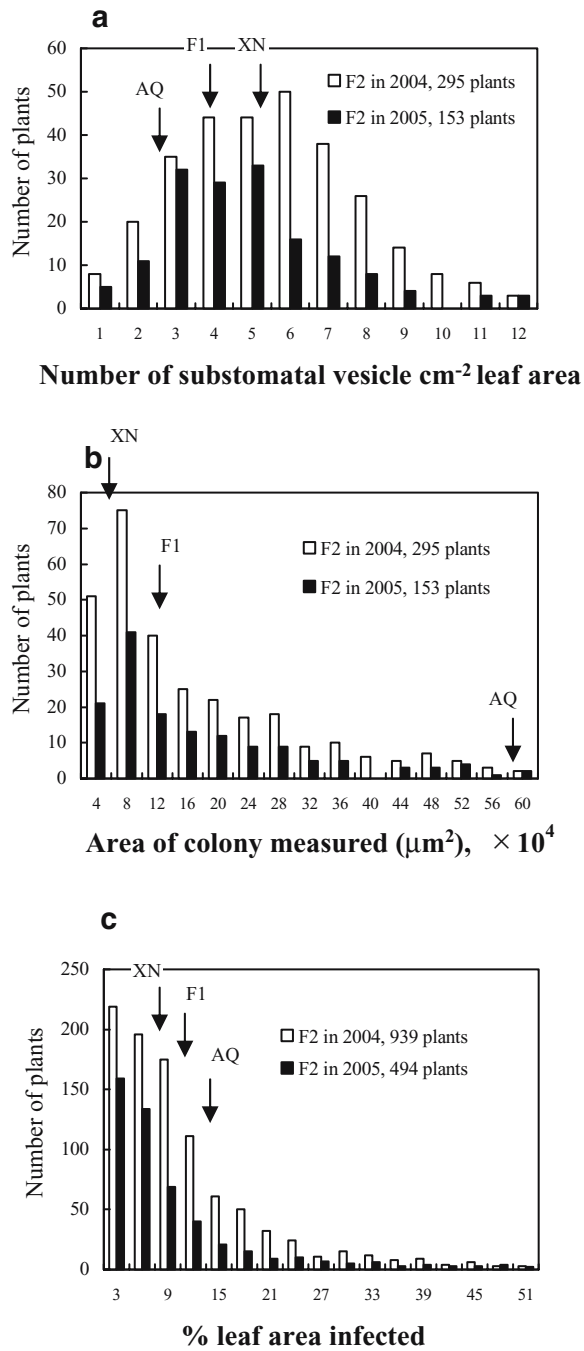

Fig. 1 Frequency distributions of F_2 plants from the cross between wheat cvs Aquileja and Xian Nong 4 tested with *Puccinia striiformis* f.sp. *tritici* in 2004 and 2005 for penetration frequency (**a**), colony size (**b**), and percent of leaf area with symptoms (**c**). Penetration frequency was determined as the number of substomatal vesicle per square centimeter of leaf area. Colony size was measured in square micrometers (μm^2) of an individual colony. '*AQ*', '*XN*' and 'F_1' indicate the means of Aquileja, Xian Nong 4, and their F_1 generations, respectively, averaged over the two experiments performed in 2004 and 2005. The susceptible control Ming Xian 169 showed mean penetration frequency, colony size, and disease severity, respectively, of 9.02 substomatal vesicles per square centimeter leaf area, 407×10^4 μm^2, and 87% leaf area infected

variances of F_2, the BC with higher value, the BC with lower value, and F_1 respectively, (Castle 1921; Cockerham 1986; Zeng et al. 1990), with the modification that phenotypic range was multiplied by heritability to reduce environmental influence. Method 2: $n = [(\mathrm{PR})h^2]^2 \Big/ \{5.33[V_{F3} - (V_{P1} + V_{P2} + V_{F1})/3]\}$, in which n=number of genes, PR=phenotypic range of F_3 family means, h^2=heritability, V_{F3}, V_{P1}, V_{P2}, and V_{F1}=variances of F_3, P_1, P_2, and F_1 respectively, (Bjarko and Line 1988; Cockerham 1986), with the modification that PR was multiplied by heritability. Method 3: $K_2 = (V_{2F3} - E_W) \Big/ \left\{ V_{VF3} - 2(V_{2F3})^2 \Big/ (n-1) - \left[(V_{VP1} + V_{VP2})/2 - 2(E_W)^2 \Big/ n_R \right] \right\}$, in which K_2=number of genes, V_{2F3}=mean variance of F_3 families, E_w=non-heritable variation within families, V_{VF3}=variance of F_3 variances, n=number of plants per F_3 family or harmonic mean of F_3 family sizes, V_{VP1} and V_{VP2}= variance of variances of P_1 and P_2 respectively, and n_R= number of plants within each replication of parents or harmonic mean of P_1 and P_2 sizes within replications, (Mather and Jinks 1982). The standard errors of the estimates on gene number were calculated using the formula of Lande (1981).

Results

Visible symptoms appeared 10 or 11 days after inoculation for the susceptible control MX, and 13 to 15 days for the quantitative resistance cvs AQ and XN depending on the different experiments performed in 2004, 2005, and 2006. Disease severity was 80–90% of leaf area with *Pst* symptoms 18 to 20 days after inoculation for MX, and 12–16% and 7–10% for AQ and XN respectively, depending on different experiments. Means and standard deviations

of the generations tested in 2005 are presented in Table 1 for penetration frequency and colony size. Comparisons among AQ, XN and MX showed that MX had the highest penetration frequency and largest colony size. AQ was better at limiting penetration frequency, whereas XN was better at restricting colony extension, agreeing with our previous report (Feng et al. 2007).

Frequency distributions for penetration frequency, colony size, and disease severity

There were no significant differences between reciprocal populations in the F_1 and F_2 generations (data not presented). Therefore, reciprocals were combined in all analyses. All of the traits studied showed continuous and uni-modal distributions in each of the generations tested of AQ, XN, F_1, F_2, BC_{AQ}, BC_{XN}, F_3, and F_4 as exemplified by F_2 shown in Fig. 1, typical of quantitative traits. The patterns of distribution were consistent between the two experiments in 2004 and 2005 for each trait. Transgressive segregation was obvious in both directions of low value and high value for penetration frequency and disease severity, while it was not obvious for colony size. Penetration frequency exhibited normal distributions; however, disease severity and colony size were skewed towards the small values of XN. The $\log_{10}$-transformations of disease severity and colony size improved normality (data not presented), and the geometric data were used in all analyses.

Phenotypic and genetic correlations between host effects on penetration frequency, colony size, and disease severity

Phenotypic correlation coefficients were significant at the $P=0.0001$ level between colony size and disease severity, significant at the 0.005 level between penetration frequency and disease severity, but not significant between penetration frequency and colony size at the 0.05 level (Table 2). Genetic correlation coefficients were significant at the 0.0001 level between colony size and disease severity, significant at the 0.05 level between penetration frequency and disease severity, and not significant between penetration and colony size (Table 2). The significant correlations were consistent between different segregating populations and between the experiments conducted in different seasons, and they were positive for both penetration frequency–disease severity combinations and colony size–disease severity combinations. The standard error values for the penetration frequency–disease severity correlation were higher than those for the colony size–disease severity correlation.

Table 2 Correlations between host effects on penetration frequency (PF), colony size (CS), and disease intensity (DI) of *Puccinia striiformis* f.sp. *tritici* in plants from the cross between wheat cvs Aquileja (AQ) and Xian Nong 4 (XN)

Generation and year	Number of plant, family	PF×DI		CS×PF		CS×PF	
		r[a]	P[b]	r	P	r	P
F_2 in 2004	293	0.293	<0.0001	0.618	<0.0001	0.064	0.2899
F_2 in 2005	153	0.302	0.0003	0.631	<0.0001	0.073	0.3882
BC_{AQ} in 2005	84	0.334	0.0019	0.575	<0.0001	−0.109	0.3529
BC_{XN} in 2005	64	0.356	0.0039	0.643	<0.0001	0.106	0.4026
F_3 in 2005	700, 70[c]	0.339	0.0040	0.617	<0.0001	0.109	0.3680
F_2/F_3 in 2004–2005	700, 70[d]	0.266±0.017	0.0173	0.563±0.008	<0.0001	−0.060±0.007	0.5690
F_3/F_4 in 2005–2006	700, 70[e]	0.281±0.019	0.0124	0.578±0.007	<0.0001	–[f]	–

[a] r=Correlation coefficient, ±standard error.

[b] P=Probability assuming $r=0$.

[c] Seventy F_3 families with ten plants from each family were observed.

[d] Seventy F_2 plants in 2004 and 70 F_3 families with ten plants from each family in 2005 were observed. F_3 families descended from F_2 plants.

[e] Five plants from each of 70 F_3 families were sampled in 2005 to produce F_4 families; 70 F_4 family groups with five families from each group and five plants from each family were observed in 2006.

[f] No data.

Inheritance of host effects on penetration frequency and colony size

We previously reported on the inheritance for disease severity (Zhang et al. 2001); therefore, analyses on inheritance were performed only for penetration frequency and colony size here. The standard deviations of non-segregating generations (i.e. parents and F_1) were lower than those of segregating generations (i.e. F_2, BC_{AQ}, BC_{XN}, and F_3), suggesting the existence of genetic variation (Table 1).

Penetration frequency data and $\log_{10}$(colony size) data (Table 1) fitted all and nine, respectively, of the 11 tested models by the χ^2 test at P=0.05 (Table 3), while only in one, $m[d]$, of the fitted models was every component significantly different from zero by the t-test at P=0.05.

The heritability values estimated by two different methods were nearly equal for colony size. For penetration frequency, however, the heritability estimated by method 1 was higher than that estimated by method 2 (Table 3). Standard error of heritability for

Table 3 Estimates on gene action, heritability, and number of genes associated with host effects on penetration frequency (PF) and colony size (CS) of *Puccinia striiformis* f.sp. *tritici* in plants from the cross between wheat cvs Aquileja (AQ) and Xian Nong 4 (XN)

Genetic item	Trait	
	PF	CS
Gene action[a]		
Tested model, χ^2 value, df[b], P for χ^2 test	m***[d]**, 0.16, 4, 0.997	m***[d]***, 2.26, 4, 0.688
	m***[h], 6.05, 4, 0.195	m***[h]**, 37.54, 4, <0.001
	m***[d]*[i], 0.11, 3, 0.991	m***[d]***[i], 0.79, 3, 0.851
	m*[h][l], 5.86, 3, 0.119	m***[h]*[l], 34.06, 3, <0.001
	m***[d]*[h], 0.13, 3, 0.988	m***[d]***[h], 1.40, 3, 0.705
	m[d]*[h][i], 0.10, 2, 0.953	m***[d]***[h][i], 0.13, 2, 0.937
	m***[d]*[h][j], 0.10, 2, 0.950	m***[d]***[h][j], 1.31, 2, 0.519
	m***[d]*[h][l], 0.12, 2, 0.940	m***[d]***[h][l], 0.17, 2, 0.918
	m[d]*[h][i][j], 0.08, 1, 0.781	m***[d]***[h][i][j], 0.03, 1, 0.854
	m[d]*[h][i][l], 0.03, 1, 0.863	m**[d]***[h][i][l], 0.10, 1, 0.751
	m***[d]*[h][j][l], 0.10, 1, 0.752	m***[d]***[h][j][l], 0.07, 1, 0.799
Accepted model	m***[d]**	m***[d]***
Estimate of m	3.818	5.249
t value for m	7.234	68.497
P for t test on m	<0.001	<0.001
Estimate of [d]	1.639	0.570
t value for [d]	2.746	6.535
P for t test on [d]	0.007	<0.001
Heritability (%)[c]		
Method 1	77	76
Method 2	62±7	74±5
Number of genes[d]		
Method 1	3.2±0.6	2.8±0.4
Method 2	3.5	2.7
Method 3	4.1	3.3

*Significant at P=0.05 for t-test.

**Significant at P=0.01 for t-test.

***Significant at P=0.001 for t-test.

[a] In the models, m mean of all homozygous individuals, [d] additive component, [h] dominance component, [i] additive×additive interaction, [j] additive×dominance interaction, and [l] dominance×dominance interaction. (17). Models having P>0.25 for χ^2 test were considered to fit the data.

[b] Degrees of freedom.

[c,d] See "Materials and methods"

penetration frequency was higher than that for colony size.

The numbers of genes estimated by three different methods were near to one another for both penetration frequency and colony size. Standard error of gene number for penetration frequency was higher than that for colony size (Table 3).

Discussion

Improvement of the estimates

For quantitative analysis, it is important to improve the accuracy and precision, and thus reliability, of an estimate (Falconer 1981; Mather and Jinks 1982). Three methods were made in the present study to achieve higher reliability. Firstly, eight generations were used including P_1, P_2, F_1, F_2, F_3, F_4, backcrossed to P_1, and backcrossed to P_2 (Tables 1 and 2). Utilization of the integrated data of these generations might increase the accuracy of estimates. Secondly, as detailed above, all of the experimental plants were artificially inoculated with a single-spore derived isolate of *Pst* at a high inoculum density, and the plants were well conditioned to facilitate infection. This should improve the uniformity of infection and decrease the chance for plants to escape from inoculum, as otherwise may occur using spreader-inoculation or natural inoculation. Finally, two or three statistical methods were applied (Tables 2 and 3) to estimate heritability or gene number. These methods are based on different genetic theories or assumptions (see Materials and methods), but the estimates by these methods are quite similar, suggesting their consistency (Tables 2 and 3). Some of these methods were reported previously (Das et al. 1993; Chen and Line 1995; Zhang et al. 2001).

Quantification of host effects on penetration frequency and colony size, and the relationship between them

No reports are available for the genetics of host effects on infection frequency and colony extension of *Pst*, while a similar study was reported for host effects on *Puccinia recondita* f. sp. *tritici* (*Prt*) by Das et al. (1993). However, *Pst* as tested in the present study is characterized by unrestricted growth of individual infections or semi-systemic development (Baum and Savile 1985; Vallavieille-Pope et al. 1995). Scattered infections may coalesce even before visible symptoms appear. Coalescence can interfere with distinguishing between individual infections, and consequently, it may influence the accuracy of quantification. We microscopically quantified the infection frequency and colony extension of *Pst* at early stages of pathogenesis when individual infections were quite distinct. This method might eliminate the influence of coalescence on the analyses.

The lack of a significant correlation between penetration frequency and colony size (Table 2) suggests that the wheat genes for limiting infection frequency of *Pst* were different from those for restricting colony extension. This would be understandable when considering that one of the parents, AQ, was characterized by its higher ability to limit infection frequency, whereas the other parent, XN, was better at restricting colony extension (Table 1). It was estimated that three or four genes, and three genes, controlling penetration frequency and colony size respectively, segregated in the cross AQ×XN, and they were transmitted with moderately high heritability (Table 3). The acceptance of only one model, $m[d]$, indicates that these genes acted in additive fashion with no detectable dominant and epistatic effects (Table 3). From the significant transgressiveness for penetration frequency (Fig. 1a), it can be inferred that the genes for penetration frequency might come from both AQ and XN. Indeed, both AQ and XN had lower penetration frequency values than the susceptible reference cultivar MX (Table 1). It seems that the genes for colony size were contributed primarily by XN since no obvious transgressiveness occurred for colony size (Fig. 1b). We also conducted an analysis on $\log_{10}$(colony size), but again no significant transgressiveness for colony size was detected (data not presented). The skewness of colony size towards small values (Fig. 1b) could be an indication that some of the genes for colony size might have larger effects on restricting colony extension than the other(s). Unequal effects of genes violate the assumption underlying the methods used for estimating gene number (Cockerham 1986; Mather and Jinks 1982; Zeng et al. 1990). This situation can cause an

underestimation of the number of resistance genes; therefore, the actual number of genes in the cross AQ× XN may be more than that estimated.

Relationships of host effects on disease severity with those on penetration frequency and colony size

Both penetration frequency and colony size of *Pst* were strongly associated with disease severity (Table 2), and this is reasonable since they are among the pathological components of disease severity. Colony size explained up to 33% of genotypic variation of disease severity, as calculated by squaring their corresponding correlation coefficients (Table 2). Penetration frequency, however, accounted for only up to 8% of the genotypic variation of disease severity. One reason for this difference might be that penetration frequency contributes less than colony size to disease severity. *Pst*, as mentioned above, is characterized by unrestricted growth. It has been reported that under optimal conditions, a single colony of *Pst* in a susceptible young wheat leaf could extend and ramify until the entire leaf surface is occupied (Mares and Cousen 1977). In the present observations, we found that a single *Pst* colony in a wheat flag leaf could extend in both directions towards the tip and base of the leaf with a coverage up to 3.7 cm in the quantitatively resistance cv. AQ, and up to 12.4 cm in the susceptible control MX (Feng et al. unpublished data). Even a few number of infections could result in symptoms on a large leaf area, and thus colony extension might contribute more than penetration frequency to disease severity.

Another reason might be that the quantification of penetration frequency was less accurate than that of colony size, and thus the correlation between penetration frequency and disease severity was attenuated. Penetration frequency was more prone to experimental errors than colony size. Thus the standard errors of penetration frequency-involved correlation coefficients, heritability, and gene number were all higher than those of colony size-involved respective estimates (Tables 2 and 3). These results would be expected considering that colony size was repeatedly measured even within a single leaf segment sample of a square centimeter (e.g. 2.2 repeats per square centimeter for AQ and 5.4 repeats per square centimeter for XN on average, Table 1), whereas penetration frequency could be measured only once for the same leaf sample. Repeated measurements might reduce errors. Similar results, that receptivity (uredinia per square centimeter) was more prone to environmental variation than uredinium size, were reported for wheat leaf rust by Das et al. (1993).

Penetration frequency and colony size collectively explained up to 41% of disease severity variation genotypically and up to 54% phenotypically, implying that components other than penetration frequency and colony size might be responsible for the remaining portions of disease severity variation.

It is noticeable that significant transgressiveness occurred for disease severity (Fig. 1c), confirming our early report (Zhang et al. 2001). It can be assumed that the transgressiveness was attributed at least partially to the recombination of genes associated with penetration frequency and colony size. Pathological components other than penetration frequency and colony size, however, might also contribute to the transgressiveness since a substantial portion of disease severity variation, as shown above, was not explained solely by penetration frequency and colony size.

In addition, the durability of *Pst* resistance in AQ and XN should be noted. Evidence for durable resistance to *Pst* in the two cultivars has been previously documented (Zhang 1995; Zhang et al. 2001). XN and AQ have been commercially-used cultivars since 1975 and 1980, respectively, in northwestern China (Zhang 1995; Zhang et al. 2001; Feng et al. 2007) where the environment is conducive to *Pst*, and 12 new *Pst* virulent races were detected from 1975 to 2003 (Wan et al. 2004). Yuan et al. (1995) inoculated each of the two cultivars separately with seven *Pst* virulent races to test race-specificity, but found that the races caused no significant difference in disease severity. XN and AQ were sown on 42,000 and 32,000 ha, respectively, in 1994 (Zhang 1995; Zhang et al. 2001). The area of both cultivars fluctuated over the last 12 years, but their *Pst* resistance remains effective (Feng et al. 2007). The use of a single *Pst* isolate in the present study would not add information on the durability of resistance; however, it provided information on the pathological components and genetics of quantitative resistance.

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References

Baum, B. R., & Savile, D. B. O. (1985). Rusts (Uredinales) of *Triticae*: Evolution and extent of coevolution, a cladistic analysis. *Botanical Journal of the Linnean Society, 91*, 367–394.

Bjarko, M. E., & Line, R. F. (1988). Heritability and number of genes controlling leaf rust resistance in four cultivars of wheat. *Phytopathology, 78*, 457–461.

Broers, L. H. M., & López-Atilano, R. M. (1996). Effect of quantitative resistance in wheat on the development of *Puccinia striiformis* during early stages of infection. *Plant Disease, 80*, 1265–1268.

Castle, W. E. (1921). An improved method of estimating the number of genetic factors concerned in the cross of blending inheritance. *Science, 54*, 223.

Chen, X., & Line, R. F. (1995). Gene number and heritability of wheat cultivars with durable, high-temperature, adult-plant (HTAP) resistance and interaction of HTAP and race-specific seedling resistance to *Puccinia striiformis*. *Phytopathology, 85*, 573–578.

Cockerham, C. C. (1986). Modification in estimating the number of genes for a quantitative character. *Genetics, 114*, 659–664.

Das, M. K., Rajaram, S., Kronstad, W. E., Mundt, C. C., & Singh, P. R. (1993). Associations and genetics of three components of slow rusting in leaf rust of wheat. *Euphytica, 88*, 99–109.

Falconer, D. S. (1981). *Introduction to quantitative genetics* (2nd ed., pp. 148–169, 281–300). New York: Longman, Inc.

Feng, J., Zhang, Z. J., Li, G. H., Zhou, Y., Wang, H. H., Guo, Q. G., et al. (2007). Components of quantitative resistance to stripe rust in five wheat cultivars and genetic distance among the cultivars. *Acta Phytopathologica Sinica 37*, 175–183.

Frey, K. J., & Horner, T. (1957). Heritability in standard units. *Agronomy Journal, 49*, 59–62.

Frye, C. A., & Innes, R. W. (1998). An *Arabidopsis* mutant with enhanced resistance to powdery mildew. *The Plant Cell, 10*, 947–956.

Johnson, R. (1984). A critical analysis of durable resistance. *Annual Review of Phytopathology, 22*, 309–330.

Johnson, R., Stubbs, R. W., Fuchs, E., & Chamberlain, N. H. (1972). Nomenclature of physiological races of *Puccinia striiformis* infecting wheat. *Transactions of the British Mycological Society, 58*, 475–480.

Lande, R. (1981). The minimum number of genes contributing to quantitative variation between and within populations. *Genetics, 99*, 541–553.

Line, R. F. (2002). Stripe rust of wheat and barley in North America: A retrospective historical review. *Annual Review of Phytopathology, 40*, 75–118.

Mallard, S., Gaudet, D., Aldeia, A., Abelard, C., Besnard, A. L., Sourdille, P., et al. (2005). Genetic analysis of durable resistance to yellow rust in bread wheat. *Theoretical and Applied Genetics, 110*, 1401–1409.

Mares, D. L., & Cousen, S. (1977). The interaction of yellow rust (*Puccinia striiformis*) with winter wheat cultivars showing adult plant resistance: Macroscopic and microscopic events associated with the resistant reaction. *Physiological Plant Pathology, 10*, 257–274.

Mather, K., & Jinks, J. L. (1982). *Biometrical genetics* (3rd ed., pp. 65–175, 315–350). New York and London: Chapman and Hall.

Milus, E. A., & Line, R. F. (1986). Gene action for inheritance of durable, high-temperature, adult-plant resistance to stripe rust in wheat. *Phytopathology, 76*, 435–441.

Nyquist, W. E. (1991). Estimation of heritability and prediction of selection response in plant populations. *Critical Reviews in Plant Sciences, 10*, 235–322.

Peterson, R. F., Campbell, A. E., & Hannah, A. E. (1948). A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Research, C 26*, 496–500.

SAS Institute (1991). *SAS language and procedures: Usage 2, version 6* (1st ed.). Cary, NC, USA: SAS Institute Inc.

Singh, R. P. (1992). Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. *Phytopathology, 82*, 835–838.

Stubbs, R. W. (1988). Pathogenicity analysis of yellow (stripe) rust of wheat and its significance in a global context. In N. W. Simmonds & S. Rajaram (Eds.), *Breeding strategy for resistance to the rusts of wheat* (pp. 23–38). Mexico DF: CIMMYT.

Vallavieille-Pope, C. de, Huber, L., Leconte, M., & Goyeau, H. (1995). Comparative effects of temperature and interrupted wet periods on germination, penetration, and infection of *Puccinia recondita* f. sp. *tritici* and *P. striiformis* on wheat seedlings. *Phytopathology, 85*, 409–415.

Wan, A., Zhao, Z., Chen, X., He, Z., Jin, S., Jia, Q., et al. (2004). Wheat stripe rust epidemic and virulence of *Puccinia striiformis* f. sp. *tritici* in China in 2002. *Plant Disease, 88*, 896–904.

Yuan, W. H., Zhang, Z. J., Feng, F., & Zeng, S. M. (1995). Identification of wheat cultivars with slow-rusting resistance to *Puccinia striiformis*. *Scientia Agricultura Sinica, 28*(3), 35–40.

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research, 14*, 415–421.

Zeng, Z. B., Houle, D., & Cockerham, C. C. (1990). How informative is Wright's estimator of the number of genes affecting a quantitative character? *Genetics, 126*, 235–247.

Zhang, Z. J. (1995). Evidence of durable resistance in nine Chinese land races and one Italian cultivar of *Triticum aestivum* to *Puccinia striiformis*. *European Journal of Plant Pathology, 101*, 405–409.

Zhang, Z. J., Yang, G. H., Li, G. H., Jin, S. L., & Yang, X. B. (2001). Transgressive segregation, heritability, and number of genes controlling durable resistance to stripe rust in one Chinese and two Italian wheat cultivars. *Phytopathology, 91*, 680–686.