Multivariate analysis of stripe rust assessment and reactions of barley in multi-location nurseries

K. Xi[1], X. M. Chen[2], F. Capettini[1], E. Falconi[3], R. C. Yang[1], J. H. Helm[1], M. D. Holtz[1], P. Juskiw[1], K. Kumar[1], J. Nyachiro[1], and T. K. Turkington[4]

[1]Alberta Agriculture and Rural Development, Field Crop Development Centre, Lacombe, Alberta, Canada T4L 1W8 (e-mail: kequan.xi@gov.ab.ca); [2]USDA-ARS, Wheat Genetics, Quality, Physiology, and Disease Research Unit and Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430, USA; [3]Programa de Cereales, Estación Experimental Santa Catalina- INIAP Panamericana Sur Km 1 Quito, Ecuador; and [4]Agriculture and Agri-Food Canada, Lacombe Research Centre, Lacombe, AB, Canada T4L 1W1.

Received 2 March 2012, accepted 25 October 2012.

Xi, K., Chen, X. M., Capettini, F., Falconi, E., Yang, R. C., Helm, J. H., Holtz, M. D., Juskiw, P., Kumar, K., Nyachiro, J. and Turkington, T. K. 2013. **Multivariate analysis of stripe rust assessment and reactions of barley in multi-location nurseries**. Can. J. Plant Sci. **93**: 209–219. A total of 1357 entries, mainly consisting of hulled two-row, hulled six-row and hulless barley, were evaluated in stripe rust nurseries at Toluca, Mexico during 2007, Quito, Ecuador during 2007 and 2008, and Pullman and Mt. Vernon, USA [Pacific Northwest (PNW)] during 2007–2009. Disease screening data for barley stripe rust resistance at multiple locations and seasons were analyzed using factor analysis (FA), principal component analysis (PCA) and analysis of variance (ANOVA). Factor analysis led to the removal of a number of disease assessment variables that had inadequate disease levels or an unsuitable rating scale. The PCA scores revealed that the two-row types of barley were generally more resistant than the six-row and hulless types. ANOVA indicated that the effect of seasonal influence on screening was small, while stripe rust susceptible and resistant barley types were differentiated significantly on mean values averaged on infection type (IT) and percentage diseased leaf area (disease severity, DS) during the 3-yr tests in multiple screening nurseries. The significant correlations in disease assessments between IT and DS suggest that either assessment can be used to replace the other without a significant loss of information regarding barley stripe rust reactions. The first principal component is a useful criterion for assessing stripe rust reactions in barley lines.

Key words: Stripe rust of barley, *Puccinia striiformis* f. sp. *hordei*, screening barley stripe rust

Xi, K., Chen, X. M., Capettini, F., Falconi, E., Yang, R. C., Helm, J. H., Holtz, M. D., Juskiw, P., Kumar, K., Nyachiro, J. et Turkington, T. K. 2013. **Analyse multivariable de l'évaluation de la rouille jaune et des réactions de l'orge dans des pépinières situées à divers endroits**. Can. J. Plant Sci. **93**: 209–219. Les auteurs ont évalué au total 1 357 variétés d'orge à deux rangs, à six rangs et à grains nus dans des pépinières de présélection de la résistance à la rouille jaune de Toluca, au Mexique (2007), de Quito, en Équateur (2007 et 2008), ainsi que de Pullman et de Mt. Vernon, dans la région nord-ouest du Pacifique, aux États-Unis (2007–2009). Les données de présélection pour la résistance de l'orge à la rouille jaune issues de nombreux endroits et prélevées à diverses saisons ont été examinées par analyse factorielle (AF), par analyse en composantes principales (ACP) et par analyse de la variance (ANOVA). L'AF entraîne le retranchement de plusieurs variables de l'évaluation en raison d'un taux d'infection inadéquat ou d'une échelle d'évaluation inappropriée. Les résultats de l'ACP révèlent que l'orge à deux rangs est généralement plus résistante que celle à six rangs ou à grains nus. L'ANOVA indique que la saison exerce peu d'influence sur la présélection, mais que les variétés d'orge sensibles et résistantes à la rouille jaune se différencient sensiblement d'après les valeurs moyennes pour le type d'infection (TI) et la proportion de la surface des feuilles touchées (gravité de la maladie, GM) relevées lors des essais de trois ans dans maintes pépinières. Les corrélations significatives entre le TI et la GM lors des diverses évaluations laissent croire que l'un pourrait remplacer l'autre sans qu'on perde une part importante des renseignements sur les réactions de l'orge à la rouille jaune. La première composante principale est un critère utile pour évaluer la réaction des lignées d'orge à la rouille jaune.

Mots clés: rouille jaune de l'orge, *Puccinia striiformis* f. sp. *hordei*, présélection pour la résistance à la rouille jaune de l'orge

Barley (*Hordeum vulgare* L.) stripe rust caused by *Puccinia striiformis* Westend. f. sp. *hordei* Eriks. is becoming common in barley breeding nurseries located in central Alberta and commercial fields in southern Alberta (McCallum et al. 2006, 2007). The use of resistant barley is an economical, effective and environmentally friendly approach for stripe rust management (Hayes et al. 2001). There is variation in stripe rust reactions based on tested barley genotypes (Brown et al. 2001; Line 2002), which may be caused by genetic resistance, environmental conditions and changes in pathogen virulence. Thus, resistance can be selected based on genetic variation in barley. Variation in pathogen

Abbreviations: FA, factor analysis; PCA, principal component analysis; IT, infection type; DS, disease severity; HTAP, high temperature, adult plant: PDSA, percentage diseased spike area; PNW, Pacific Northwest

virulence, however, is known to overcome stripe rust resistance in barley (Rossi et al. 2006) and in wheat (*Triticum aestivum* L.) (Puchalski and Gaudet 2011). Traditional breeding and field tests have an essential role in developing commercial cultivars with durable resistance (Stuthman et al. 2007). High temperature, adult plant (HTAP) resistance is one type of durable resistance in barley to stripe rust (Yan and Chen 2008).

It is common practice to use infection type (IT) to measure rust reactions (Roelfs et al. 1992), as an IT can be an indicator of the compatible (susceptible) or incompatible (resistant) host-pathogen interaction (Roelfs 1988). Thus, IT primarily characterizes major gene(s) resistance specific to pathogen virulence. The degree of resistance to cereal rusts is assessed by examining the diseased area of plants (disease severity, DS) (Roelfs et al. 1992). Disease severity is used to measure quantitative resistance that is considered to be essential for selecting durable resistance. Although there is no documented information regarding the relative importance of IT versus DS in relation to the assessment of stripe rust reaction of barley, the two measurements are thought to correlate for stripe rust of wheat and barley (X. M. Chen, personal communication). In addition to the different disease scales used, disease may be assessed several times per growing season. Conducting multiple disease assessments is a general practice in epidemiological studies where the onset, development and the terminal levels of disease are measured and integrated for analysis. However, it is unknown if there are differences in barley resistance or susceptibility when assessed at early versus later dates during a growing season.

Breeding for barley resistance to stripe rust is underway at the Alberta Agriculture and Rural Development, Field Crop Development Centre (FCDC), located in Lacombe, Alberta. A large number of barley breeding and germplasm lines from the FCDC have been screened in international stripe rust nurseries since 2007. Information on the difference in stripe rust reactions between barley types is scarce, although more two-row barley appears to have been used for mapping stripe rust resistance genes than other barley types (Hayes et al. 2001). It was observed that at the FCDC Lacombe breeding site located in central Alberta two-rowed lines tended to be more resistant than other barley types (J. Nyachiro, personal communication), and that a six-rowed cultivar was found to be severely diseased while a two-rowed cultivar was virtually free of stripe rust in the same commercial field of southern Alberta (K. Xi, personal communication).

The objectives of this study were: (1) to compare the relative importance and determine the association between the two measurements (IT and DS) and two dates (early and later) of assessment per season, (2) to determine if there were differences in barley reactions over the multiple years to provide baseline information on resistance to barley stripe rust, and (3) to investigate if there were significant differences in stripe rust reactions in barley type and if the factor analysis (FA) and principal component analysis (PCA) procedures can be used to evaluate multiple disease variables so that resistant lines can be relatively easily identified.

MATERIALS AND METHODS

Barley Materials

The entries screened in the multi-nurseries included three types of barley: hulled two-row, hulled six-row, hulless barley of both row types (Table 1). The three types are referred as two-row, six-row and hulless barley thereafter in this paper. These materials primarily consisted of advanced lines for progressive yield trials from the first year (Yield 1) to the sixth year (Yield 6). A small number (approximately 30) of commercial barley cultivars was evaluated as checks in the nurseries with the majority of them being released from the breeding program of FCDC. The breeding process for the screened materials followed a modified bulk breeding method (J. Nyachiro, personal communication). After the original cross was made, the F_1 seeds produced from each cross were grown to generate F_1 plants producing F_2 seeds. These seeds were used to grow the F_2 Nursery at the FCDC breeding nurseries, near Lacombe, AB. The F_3 generations were grown under irrigation at the University of California (Davis) Desert Research Station located near the city of El Centro in southern California. From the F_3 Nursery, 500 heads (spikes) from each population were selected and bulked for advancing to F_4 generations. The F_4 to F_6 bulk populations were grown and advanced in the field at FCDC, Lacombe, AB. Approximately one to two hundred heads from each population, depending on the cross, were selected from F_6 bulk and grown out as individual F_7 head rows in the field. From each of the head row populations about 20 to 25% of the head rows were selected to form seed of the first (Yield 1) test in non-replicated yield trials. The selected lines were further tested in replicated-plots advancing from Yield 1 to Yield 6 in multi-locations across Alberta and western Canada. This was followed by 2-year co-op tests in multi-locations across western Canada before a candidate line was registered as a commercial cultivar. Prior to Yield 1, a screening was made for seed quality based on the near-infrared spectroscopy method. Screening for resistance to various diseases other than stripe rust was conducted during the advancement of the F_2 to F_6 bulks, yield and the co-op trials.

Screening Nurseries

The screening nurseries were located at Toluca (lat. 19°20′13″N, long. 99°33′57″W), Mexico, ICARDA/CIMMYT (2007), EE Santa Catalina (lat. 00°22′S, long. 78°33′W), Quito, Ecuador (2007 and 2008) and Pullman (lat. 46°43′57.36″N, long. 117°10′18.48″W) and Mt. Vernon (lat. 48°25′12″N, long. 122°19′34″W)

Table 1. Statistics of infection type and disease severity for stripe rust (*Puccinia striiformis* f. sp. *hordei*) reaction in barley by type and selected resistant lines in multi-location nurseries, 2007–2009

			Infection type			Disease severity		
year	type	N	mean	std	min–max	mean	std	min–max
2007	Hulless	122	5.5	2.0	0.0–8.0	35.4	18.2	0.0–84.0
	Six-row	180	5.8	2.1	0.0–8.0	32.4	18.0	0.0–66.0
	Two-row	209	3.0	2.2	0.0–8.0	14.1	15.9	0.0–57.0
	Total N	511						
2008	Hulless	161	5.4	1.7	1.2–8.0	40.7	18.3	0.5–68.8
	Six-row	145	6.0	1.4	1.0–8.0	47.5	12.1	0.5–72.5
	Two-row	263	3.8	1.6	1.2–8.0	18.7	18.4	0.5–57.8
	Total N	569						
2009	Hulless	60	6.0	2.1	1.0–8.0	36.8	18.0	1.0–77.5
	Six-row	87	5.8	2.3	1.0–8.0	36.1	21.7	3.0–75.0
	Two-row	130	3.4	1.7	1.0–8.0	14.3	10.1	2.0–57.5
	Total N	277						

Statistics of selected lines by barley type based on PC1 score of −2 evaluated in multi-location nurseries during 2007–2009

			Infection type			Disease severity		
type	N	%[z]	mean	std	min–max	mean	std	min–max
Hulless	32	9.3	1.8	0.9	0–3.6	3.1	3.0	0–11.0
Six-row	30	7.3	1.3	0.9	0–3.0	2.6	2.0	0–7.0
Two-row	251	41.7	1.8	0.9	0–4.0	2.7	2.5	0–12.2
Total N	313							

[z]Percentage of selected resistant lines in total number of lines of each barley type.

(USA in the Pacific Northwest, PNW) (2007–2009) (Table 1). Approximately 5 g of seeds/entry were planted in two 1-m-long row plots. Spreader rows of a highly susceptible six-row barley cv. Steptoe were seeded and inoculated using urediniospores of *P. striiformis* f. sp. *hordei* at the early tillering stage at the Pullman location and no inoculation was done at the Mt. Vernon location. For the Toluca nursery, spreader rows consisting of highly susceptible Australian two-row and American six-row barley genotypes were planted 2 wk before seeding of the test lines to ensure early infection. For the Quito nursery, two row cultivars Clipper and Shyri 2000 were seeded as spreaders. Entries were planted in two 1-m-row plots, with 0.20 m between rows with a seeding rate of approximately 100 kg ha^{-1}.

In all nurseries, a modified Cobb scale of 0–100% was used to assess disease severity (DS) on the leaf where 0% = no symptoms and 100% = maximum symptoms (Peterson et al. 1948). In this scale, a rating of 10% or less is likely considered to be a resistant reaction. A rating scale of 0–9 was used for recording infection type (IT) on the leaf, where 0 = no symptoms and 9 = abundant sporulation without necrosis or chlorosis. In this scale, ratings between 0 and 3 are considered to be resistant reactions; 4 to 6 are intermediate and 7 to 9 are susceptible (Line and Qayoum 1991). In addition to the assessments for IT and DS, percentage diseased spike area (PDSA) was used to assess stripe rust severity on barley spikes in the Quito nursery in 2009. Stripe rust assessment was conducted once per season in the Pullman nursery and twice per season for the rest of nurseries. A single assessment for the Pullman nursery was typically done between the milk (GS 75) (Zadoks et al. 1974) and the soft dough stage (GS 85). For the rest of nurseries, the first assessment was conducted between the jointing stage (GS 31–33) and the late boot stage (GS 49), and the second assessment was made at GS 85 approximately 3 to 4 wk later.

Data Analysis

Factor analysis and PCA were used to select variables from each year's data that had adequate disease levels and similar rating scales based on the proportion of eigenvalues in the total standardized variance. The principal axis method was then used to extract the components followed by an orthogonal rotation (Hatcher and Stepanski 2005). PCA was further employed to analyze the retained variables from the previous analysis in an attempt to characterize disease assessment data regarding location and assessing sequence based on the IT and DS scales. PCA and FA were carried out using SAS PROC PRINCOMP and SAS PROC FACTOR, respectively (SAS Institute, Inc. 2010).

In PCA, a correlation matrix among the disease variables each year was used to derive a small number of linear combinations (principal components, PCs) that captured as much of the information in the original disease variables as possible. The general formula for computing scores on the first PC (PC1) is written

mathematically as follows (Madden and Pennypacker 1979):

$$C_1 = b_{11}(x_1) + b_{12}(x_2) + \ldots b_{1p}(x_p)$$

where C_1 is the score on the principal component 1, b_{1i} is the coefficient of regression of principal component 1 on the ith disease variable x ($i = 1, 2, \ldots, p$). The subsequent principal components were computed in the same fashion.

The relationship between a disease variable and a PC was described in terms of the correlation between them or a loading. Following Fisher (1925) and Stevens (1986), the significance of the loading was evaluated by z-transformation, $z = [\ln(1+r) - \ln(1-r)]/2$ such that the new variable z, under the null hypothesis of no correlation or zero loading, would be distributed normally with mean being zero and variance being approximately given by $(n-3)^{-1}$ for a sample size of n individuals. Given the sample size ($n = 511$ for 2007, 569 for 2008 and 277 for 2009) in the present study (Table 1), a statistically significant loading may or may not be practically important (Stevens 1986). Following Stevens' suggestion (1986, p. 345), we used a more stringent threshold of 0.4 below which an observed loading was considered practically insignificant. While this threshold is arbitrary, it allows a disease variable to share at least 15% of its variance with the PCs and helps reduce the rate of false positives.

The retained variables from the previous PCA in IT and DS were each used for the analysis of variance (ANOVA) to determine statistical differences in stripe rust reaction among year and barley type within year using the following linear model of Yang et al. (1997):

$$D_{ijl} = \mu + S_i + T_{j(i)} + e_{ijl}$$

where D_{ijl} is DS or IT of the lth lines of the jth barley type in the ith season, μ is the overall mean, S_i is the effect of ith season, $T_{j(i)}$ is the effect of the jth type within the ith season, and e_{ijl} is the residual representing line to line variability within type. It should be noted that the line effect was not tested with this linear model because line was unreplicated in this screening experiment. All effects except for μ were considered random and unrelated. The analysis was done using SAS PROC MIXED (SAS Institute, Inc. 2010) and the likelihood ratio method was used according to Littell et al. (2002).

Finally, the PCA scores were plotted using SAS PROC PLOT (SAS Institute, Inc. 2010) for individual observations to gain insight into stripe rust reactions among barley types. The mean IT and DS values were obtained by averaging the multiple IT and DS ratings, respectively, each year and a correlation analysis was used to measure the associations between mean IT and DS measurement in relation to principal components. Resistant lines were, then, selected based on the score of the major principal component (s) on which IT and/or DS had high loadings.

RESULTS AND DISCUSSION

The correlation coefficients between each stripe rust measurement and their scores on each of the first three PCs are presented in Table 2. In interpreting the component pattern, a disease assessment was considered to load on a given component if the loading in a correlation value was 0.4 or greater for that component (Hatcher and Stepanski 2005). The variables that had a loading of 0.4 or greater on more than one component were removed from further analyses, as they were not pure measures of any one construct of variables (Hatcher and Stepanski 2005). Based on the correlation of 0.4 criterion, the use of FA and PCA identified five variables to be redundant and they were removed for subsequent analyses (Table 2). The five variables consisted of one IT rating at Mt. Vernon in 2007 (Variable 3), one DS rating at Pullman in 2008 (Variable 2), one IT and one DS at Pullman in 2009 (Variables 1 and 2), and a PDSA rating at Quito in 2009 (Variable 11) (Table 2). Examination of the raw data indicated that the removed variables were relatively low in either or both IT and DS assessed within a nursery (Table 2). The exact biological reason for the PDSA data to be identified as redundant variables and removed is unclear. However, the PDSA scale has not been commonly used for the measurement of stripe rust severity on barley spikes. In the evaluation of screening data, it is a common practice to empirically remove disease assessment data with low levels of disease or no disease. Coincidentally, the current analyses resulted in statistically removing the low disease severity data that would have been removed empirically without the use of FA and PCA procedures.

A correlation matrix based on the retained variables was used for PCA of the 2007 data (Table 3a). This matrix showed that there were generally higher correlations in the IT and DS assessment between the two dates for the Toluca than the Mt. Vernon nursery. Subsequent PCA showed that the first three components for disease assessments displayed eigenvalues greater than 4.6, 1.5 and 0.9, respectively, accumulatively accounting for 78% of total variance (Table 4a). Therefore, only these three components were retained for interpretation. The PCA results for 2007 showed that the first eigenvector had positive weights on all variables with larger weights on the four Toluca measurements (bold numbers in Table 4a). The first PC (PC1) appeared to be a measure of overall IT and DS with a larger differentiation of disease reactions being displayed. The second eigenvector had high positive weights on the IT and DS rating for Pullman and the first DS rating for Mt. Vernon (bold numbers). This contrasts against relatively small and negative weights to the remaining eigenvectors including the second rating in Mt. Vernon and all those in Toluca. The third eigenvector had relatively larger positive weights (bold numbers) on the two second date assessments that accounted for maximum variance in IT and DS in the Mt. Vernon nursery. The variables for all assessments in the Toluca nursery were loaded into the

Table 2. Correlation coefficient between each disease measurement and their scores on the first three PC in each year

Disease assessment variable	PC1	PC2	PC3	Variable mean
2007				
1. IT[z] rating in Pullman (PIT)	0.31	0.71*	−0.07	1.5
2. DS[z] rating in Pullman (PDS)	0.21	0.70*	−0.20	2.6
3. 1st IT rating in Mt. Vernon (MIT1)	0.03	0.70*	0.46*	3.1
4. 1st DS rating in Mt. Vernon (MDS1)	0.05	0.82*	0.27	3.5
5. 2nd IT rating in Mt. Vernon (MIT2)	0.38	0.08	0.77*	6.3
6. 2nd DS rating Mt. Vernon (MDS2)	0.34	0.01	0.77*	46.9
7. 1st IT rating in Toluca (TIT1)	0.85*	0.16	0.32	5.1
8. 1st DS rating in Toluca (TDS1)	0.88*	0.23	0.13	30.1
9. 2nd IT rating in Toluca (TIT2)	0.81*	0.13	0.35	3.4
10. 2nd DS in Toluca (TDS2)	0.92*	0.19	0.22	39.9
2008				
1. IT rating in Pullman (PIT)	0.33	0.14	0.50*	3.7
2. DS rating in Pullman (PDS1)	−0.05	0.53*	0.46*	1.9
3. 1st IT rating in Mt. Vernon (MIT1)	0.12	0.06	0.57*	5.6
4. 1st DS rating in Mt. Vernon (MDS1)	−0.05	−0.08	0.74*	2.5
5. 2nd IT rating in Mt. Vernon (MIT2)	0.83*	0.02	0.11	6.9
6. 2nd DS rating Mt. Vernon (MDS2)	0.87*	0.17	0.11	64.3
7. 1st IT rating in Quito (QIT1)	0.40	0.77*	−0.10	2.3
8. 1st DS rating in Quito (QDS1)	0.19	0.88*	0.08	5.9
9. 2nd IT rating in Quito (QIT2)	0.88*	0.19	0.07	5.5
10. 2nd DS in Quito (QDS2)	0.86*	0.30	0.08	58.0
2009				
1. IT rating in Pullman (PIT)	0.49*	0.58*	0.02	4.6
2. DS rating in Pullman (PDS)	0.55*	0.51*	−0.02	9.8
3. 1st IT rating in Mt. Vernon (MIT1)	0.13	0.25	0.82*	5.2
4. 1st DS rating in Mt. Vernon (MDS1)	0.16	0.04	0.87*	15.9
5. 2nd IT rating in Mt. Vernon (MIT2)	0.03	0.84*	0.25	6.8
6. 2nd DS rating Mt. Vernon (MDS2)	0.30	0.76*	0.36	55.2
7. 1st IT rating in Quito (QIT1)	0.81*	0.27	0.22	3.0
8. 1st DS rating in Quito (QDS1)	0.89*	0.15	0.11	14.0
9. 2nd IT rating in Quito (QIT2)	0.68*	0.11	0.25	3.9
10. 2nd DS in Quito (QDS2)	0.90*	0.22	0.13	17.9
11. Percentage diseased spike area in Quito (QDSA)	0.44*	0.44*	−0.13	5.1

[z]IT, infection type; DS, disease severity = percentage diseased leaf area.
*Meaningful loading ≥0.4.

same component (PC1). This suggested that the early and late assessment data during the season were closely related in the levels of stripe rust due to the similar differential disease levels for individual barley genotypes between the two assessments. The separation of the two date assessments using the DS scale for the Mt. Vernon variables into the second and third components suggests that the lack of correlation between the two date assessments reflect the change in disease responses between the early and late growing season, which is possible due to the expression of high temperature adult plant (HTAP) resistance as a type of durable resistance to stripe rust. The close correlations in disease ratings between the first and second assessments for the Toluca nursery were confirmed with substantially high correlation coefficients, while the lack of correlation between the assessments was evidenced by substantially low coefficients for the Mt. Vernon nursery (Table 3a). An assessment using either average IT or DS based on the mean across multiple ratings in each year was highly significantly correlated with PC1, but not with PC2 or PC3 (Table 3d). The high correlation with PC1 was not surprising as PC1 accounted for a large amount, while PC2 and PC3 each accounted for a relatively small amount of total variance in the IT and DS assessments. There was a close correlation ($r = 0.85^{**}$) in a mean disease assessment between the two scales used for 2007.

With regard to the analyses for the 2008 data, a correlation matrix showed that there were generally higher correlations in the IT and DS assessments between the two dates for the Quito than the Mt. Vernon nursery (Table 3b). These three components displayed eigenvalues greater than 1 and accumulatively accounted for approximately 70% of the total variance with PC1 accounting more variance (44%) than other two PCs (13% each) (Table 4b). The first eigenvector had positive weights on all variables with slightly more weighting on the second assessments than those for the first ones in Mt. Vernon and Quito (bold numbers in Table 4b), suggesting that the second date assessment near the end of season was more effective, than the first date of assessment before the soft dough stage, in differentiating stripe rust reactions of barley lines in the two nurseries. The eigenvectors in the second PC had larger positive

Table 3a. Correlation matrix among different measurements used for principal component analysis for 2007

Variable	PDS	MDS1	MIT2	MDS2	TIT1	TDS1	TIT2	TDS2
PIT	0.47	0.45	0.21	0.15	0.32	0.38	0.29	0.37
PDS1		0.42	0.11	0.09	0.18	0.29	0.16	0.24
MDS1			0.23	0.17	0.30	0.30	0.29	0.29
MIT2				0.66	0.52	0.45	0.55	0.52
MDS2					0.49	0.41	0.48	0.46
TIT1						0.79	0.85	0.83
TDS1							0.68	0.91
TIT2								0.83

Table 3b. Correlation matrix among different measurements used for principal component analysis for 2008

Variable	MIT1	MDS1	MIT2	MDS2	QIT1	QDS1	QIT2	QDS2
PIT1	0.10	0.12	0.28	0.32	0.15	0.20	0.30	0.36
MIT1		0.19	0.14	0.16	0.13	0.14	0.13	0.17
MDS1			0.02	0.02	−.03	0.06	0.04	0.05
MIT2				0.74	0.33	0.22	0.59	0.58
MDS2					0.43	0.33	0.71	0.72
QIT1						0.69	0.46	0.55
QDS1							0.32	0.40
QIT2								0.92

Table 3c. Correlation matrix among different measurements used for principal component analysis for 2009

Variable	MDS1	MIT2	MDS2	QIT1	QDS1	QIT2	QDS2
MIT1	0.62	0.33	0.44	0.32	0.24	0.30	0.28
MDS1		0.19	0.35	0.29	0.21	0.29	0.25
MIT2			0.74	0.35	0.26	0.38	0.30
MDS2				0.55	0.46	0.59	0.51
QIT1					0.75	0.87	0.77
QDS1						0.65	0.92
QIT2							0.76

Table 3d. Correlation coefficients between mean trait values and principal component scores for each of the 3 yr

		Mean IT			Mean DS		
Year	IT/DS	PC1	PC2	PC3	PC1	PC2	PC3
2007	0.85**	0.96**	−0.01	0.01	0.96**	−0.07	0.04
2008	0.86**	0.94**	0.17**	0.05	0.97**	−0.04	−0.06
2009	0.85**	0.96**	0.10	−0.05	0.96**	−0.06	−0.05

**Significant at $P = 0.01$.

weights on the first three assessments for Pullman and Mt. Vernon (bold numbers in Table 4b). The IT assessment from Pullman probably reflected a low disease level that accounted for a small variance of the disease variable. The weights loaded in PC2 with the first IT and DS assessments from Mt. Vernon also reflected the relatively lower disease levels at the early stage of barley growth in comparison with the disease levels measured at the later growth stage. The remaining assessments in PC2 including the second rating in Mt. Vernon and all ratings at both growth stages in Quito had smaller and negative weights. The third component had relatively larger and positive weights on the first assessments using IT and DS in Quito (bold numbers in Table 4b), indicating the importance of the second rating at the later growth stage of barley relative to the first rating at the early stage in this nursery, given that the two date assessments were loaded on PC1 and PC3, respectively. There was a close correlation ($r = 0.86$**) in disease assessment based on the mean

Table 4a. Eigenvectors on the first three principal components for each of the measurements in 2007

Location, rating scale and rating sequence	PC1	PC2	PC3
% PC	51	17	10
Pullman, IT (PIT)	0.24	**0.51**	0.02
Pullman, DS (PDS)	0.17	**0.58**	0.11
Mt. DS, 1st (MDS1)	0.22	**0.48**	0.24
Mt. IT, 2nd (MIT2)	0.32	−0.23	**0.54**
Mt. DS, 2nd (MDS2)	0.29	−0.27	**0.60**
Toluca, IT, 1st (TIT1)	**0.42**	−0.14	−0.23
Toluca, DS, 1st (TDS1)	**0.40**	−0.02	−0.33
Toluca, IT, 2nd (TIT2)	**0.40**	−0.16	−0.17
Toluca, DS, 2nd (TDS2)	**0.43**	−0.09	−0.30

Table 4b. Eigenvectors on the first three principal components for each of the measurements taken in 2008

Location, rating scale and rating sequence	PC1	PC2	PC3
% PC	44	13	13
Pullman, IT (PIT)	0.22	**0.25**	−.20
Mt. IT, 1st (MIT1)	0.13	**0.59**	0.12
Mt. DS, 1st (MDS1)	0.04	**0.74**	0.01
Mt. IT, 2nd (MIT2)	**0.38**	−.05	−.33
Mt. DS, 2nd (MDS2)	**0.43**	−.06	−.23
Quito, IT, 1st (QIT1)	0.35	−.14	**0.55**
Quito, DS, 1st (QDS1)	0.29	−.01	**0.66**
Quito, IT, 2nd (QIT2)	**0.44**	−.08	−.19
Quito, DS, 2nd (QDS2)	**0.46**	−.06	−.09

Table 4c. Eigenvectors on the first three principal components for each of the measurements taken in 2009

Location, rating scale and rating sequence	PC1	PC2	PC3
% PC	55	17	13
Mt, IT, 1st (MIT1)	0.25	**0.55**	0.29
Mt, DS, 1st (MDS1)	0.23	**0.51**	0.49
Mt, IT, 2nd (MIT2)	0.28	0.30	**−.67**
Mt, DS, 2nd (MDS2)	0.37	0.23	**−.42**
Quito, IT, 1st (QIT1)	**0.42**	−.22	0.09
Quito, DS, 1st (QDS1)	**0.39**	−.34	0.15
Quito, IT, 2nd (QIT2)	**0.41**	−.18	0.02
Quito, DS, 2nd (QDS2)	**0.41**	−.31	0.13

across multiple rating variables between the IT and DS scale for 2008 (Table 3d). Furthermore, the PC1 score was highly significantly correlated with the mean across multiple rating assessments based on either IT or DS. Despite of its significance, PC2 had a very low correlation coefficient (0.17**) with mean IT. PC3 was not significantly correlated with either IT or DS assessment.

A correlation matrix based on the retained variables of the 2009 data was used for PCA (Table 3c). The considerably higher correlations for the two dates of assessment in Quito compared with those correlations in Mt. Vernon suggest that disease levels were closely related between the two growth stages in Quito. The considerably lower correlation coefficients between the first and second assessments in the Mt. Vernon nursery suggest there were changes in the ranking of disease levels among genotypes between the two growth stages at this site. The three components each had eigenvalues greater than 1, accounting for 85% of the total variance, and appeared to explain the weight of eigenvalues in relation to disease measurements in each nursery (Table 4c). The first eigenvector had similar positive weights on all variables with larger weights for the Quito than for the Mt. Vernon nursery (bold numbers in Table 4c), suggesting that PC1 is a measure of overall IT and DS with a larger differentiation of disease reactions for the Quito nursery. The second and the third eigenvectors each had more weight on the first and second assessments in the Mt. Vernon nursery, respectively, displaying larger variance for disease reactions than the remaining assessments (bold numbers in Table 4c). There was a close correlation ($r = 0.85$**) in mean disease assessment between the IT and DS scales for 2009 (Table 3d). A mean assessment based on either IT or DS was highly significantly correlated with PC1, but not with PC2 or PC3.

In each of 3 yr, the hulled two-row type was found to be considerably lower in mean IT and DS over multiple assessment data than the hulled six-row and hulless types (Table 1), indicating that the two-row type was more resistant to stripe rust than other two types of barley. ANOVA showed that the differences were significant among barley type for both mean values of IT and DS (Table 5). While the year difference was not significant for IT, it was for DS. However, the likelihood ratio for the year effect was considerably smaller than that for the effect of barley type in DS. The fact that the effect of barley type variance was much larger than the effect of year variance for both IT and DS suggests that environmental conditions during the 3 yr had a relatively small influence on the observed disease resistance or susceptibility, and the entries were differentiated by barley type for stripe rust reactions.

As multivariate analysis procedures, FA and PCA consider screening data from multiple seasons and nurseries simultaneously instead of analyzing one variable at a time. Thus, it is possible to relatively easily identify the trends of susceptibility or resistance for barley types on the plot of the first three principal components after data reduction. Based on the 2009 data, PCA tended to separate barley types in stripe rust reaction in a way that would be expected. With the first two principal components, the majority of six-row and hulless lines with high IT and DS can be found at the right and the majority of the hulled two-row lines with low IT and DS are at the left in the plot (Fig. 1). It is also possible to identify the same trends for stripe rust reaction in barley types based on the plot of the first and third principal components. Similarly to the plot of the first two components, very few hulled six-row and hulless lines are at the left and very few hulled two-row lines are at the right (Fig. 2). Both plots based on the first

Table 5. Analysis of variance to test the significance of the year and barley type effects on the observed stripe rust infection type (IT) and percentage diseased leaf area (disease severity, DS)

Infection type (IT)

Model	−2 Residual log likelihood	Likelihood ratio statistics	$Pr > \chi^2$
Full model	5680.8		
Model without barley type (year)	6057.6	377.6	<0.0001
Model without barley type and year	6057.6	0	1

Disease severity (DS)

Model	−2 Residual log likelihood	Likelihood ratio statistics	$Pr > \chi^2$
Full model	11509.1		
Model without barley type (year)	11955.8	446.7	<0.0001
Model without barley type and year	11981.8	26.0	<0.0001

three principal components showed that the separation of barley types for stripe rust reaction is possible but not absolute due to somewhat overlapping in disease reactions among barley types. The PCA plots for the 2007 and 2008 season (data not shown) displayed similar patterns in separating barley types for stripe rust resistance to those using the 2009 data. However, over 5000 observation points for each of the 2007 and 2008 seasons resulted in many hidden values in the PCA plots as opposed to over 2000 points for the 2009 plot that greatly reduced the number of hidden observations and thereby improved the clarity of presentation. These results are not unexpected as lines tested in earlier years are being advanced although in lower numbers to subsequent nurseries.

Since both IT and DS assessments had high loadings on PC1 compared with PC2 and PC3, we selected resistant lines based on PC1 scores (C_1) that were equal to or less than −2. This resulted in the selection of 313 lines from a total of 1357 entries for the 3-yr screening (Table 1). Of a total 277 lines screened for the 2009 season, 55 lines that have been selected based on the −2 score of PC1 were found to be distributed in the extreme left of each plot (Figs. 1 and 2). Further examination showed that the majority (95%) of the selected 313 lines for the 3-yr screening based on the −2 score of PC1 range from 0 to 3 in IT and from 0 to 10% in DS (Table 1). Thus, the selected lines were considered to be resistant and the selection based on a −2 score was effective. In comparison of the number of lines selected in their respective barley type, considerably more two-row type lines (41.7%) than the hulless (9.3%) and six-row type lines (7.3%) were selected based on the −2 of PC1 score

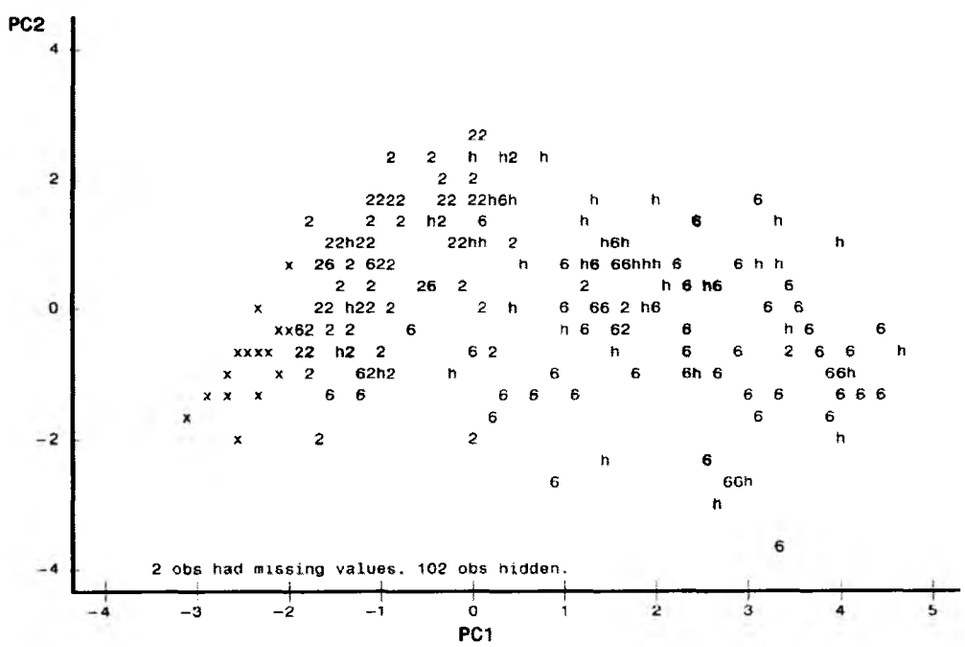

Fig. 1. Plot of genotypic scores for the first two principal components based on stripe rust infection type (IT) and disease severity (DS) by barley type from Pullman, Mt. Vernon and Quito in 2009. Symbol is value of entry where 2 = hulled two-row barley, 6 = hulled six-row barley, h = hulless barley, and x = selected resistant lines.

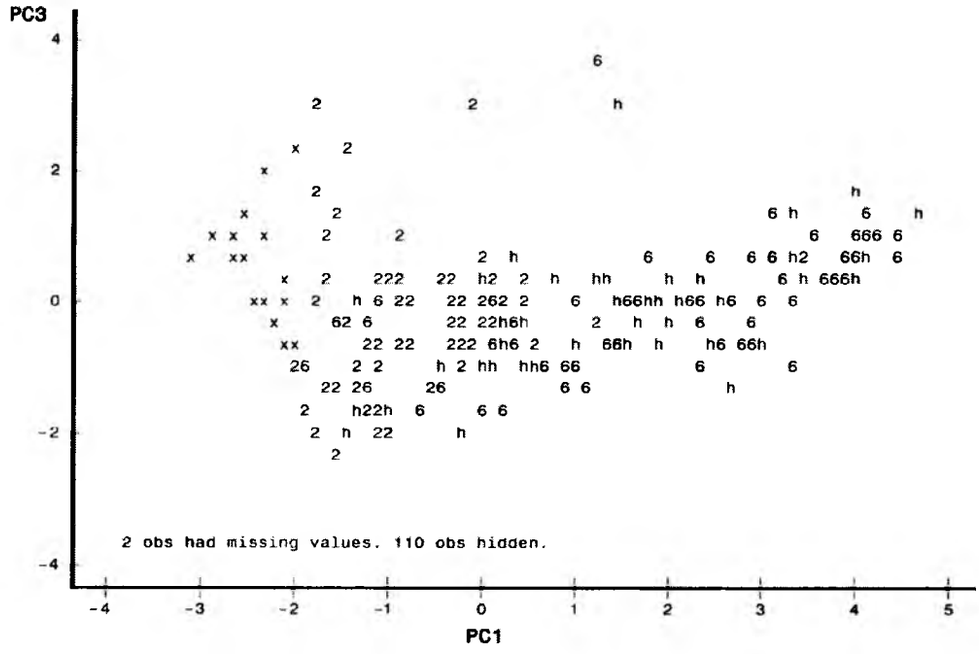

Fig. 2. Plot of the genotypic scores for the first and the third principal components based on stripe rust infection type (IT) and disease severity (DS) by barley type from Pullman, Mt. Vernon and Quito in 2009. Symbol is value of entry where 2 = hulled two-row barley, 6 = hulled six-row barley, h = hulless barley, and x = selected resistant lines.

(Table 1). The fact that the selected lines of all types of barley met the resistant criteria in both IT and DS measurements suggests a close association between the two scale assessments within the range of selection. Moreover, close associations between the IT and DS ratings were demonstrated by retaining the variables in 12 pairs regardless of season or nursery (Tables 4a–c). The data based on the IT and DS scales were also found to be similar in weights and were loaded in the same component (Tables 4a–c), indicating the similarity in the degree of differentiation of disease reactions between the two scales used. Although cereal rusts, including stripe rust of barley, are typically assessed using both IT and DS (Roelfs et al. 1992), the close relationship between the two scales suggests that to save time and labor in a field assessment, either type of assessment will be adequate without sacrificing too much information on barley disease reactions when data range is similar to that in the present study (Table 1). The resistant lines with desirable levels of disease reaction can, thus, be selected based on a range of the PC scores when there is a close correlation between the PC scores and assessment data using either scale.

The PCA results may provide insight into the usefulness of multiple disease assessments per season within a nursery. The degree of correlations indicative of the relative consistence between the two date assessments was confirmed in the correlation matrices (Tables 3a–c). This analysis separated the two dates of assessments per season into different components in each of three seasons in Mt. Vernon, in one of two seasons in Quito and none in Toluca (Tables 3a–c), suggesting that the differential levels in barley reactions to stripe rust between the two growth stages might have been caused by the expression of HTAP resistance in Mt. Vernon, whereas the environmental conditions during the growing season in other nurseries such as Toluca, Mexico, and Quito, Ecuador, might not have strongly affected host genetics for the expression of stripe rust resistance. The close correlations in disease levels between the two date assessments in the Toluca and Ecuador nursery may, thus, indicate consistence in barley disease reaction between the late vegetative and grain-filling stages, resulting in a reliable selection for that trait. Nevertheless, the separation of the two date assessments was shown to be nursery dependent. This suggests the necessity of screening in multiple nurseries to account for the variation due to changes in nursery conditions. Pathogen race variation or differences in visual rating by different raters might also have contributed to variation in disease assessment. *Puccinia striiformis* f. sp. *hordei* consists of a heterogeneous population in North America (Chen 2007; Brown et al. 2001; Kumar et al. 2012) and the complexity in race population can likely be found across the entire area where the nurseries are located. In addition, visual disease assessment may have resulted in variation among

the screening nurseries. Variation in accuracy and precision of disease assessment is recognized in powdery mildew on apple (Seem and Gilpatrick 1980) and different raters are known to cause variation in quantification of disease reactions of northern leaf blight in corn (Poland and Nelson 2011).

Information on genetic resistance in the FCDC barley lines screened in the nurseries is largely unavailable except for disease reactions in barley type and phenotype that were differentiated in the present study. Coincidentally, research including mapping populations of barley has been carried out with more two-row than six-row barley types (Hayes et al. 2001; Rossi et al. 2006). Furthermore, one of the most widely grown cultivars in the United States is a two-row feed cv. Baronesse with a moderate level of non-race-specific HTAP resistance (Chen 2007). Given that the source of multiple disease resistance in the FCDC's barley breeding program was obtained through collaboration with Dr. Hugo Vivar of ICARDA/CIMMYT (Helm 2001), genetic resistance in the FCDC lines may be similar to that in two-row barley cv. Seebe from the FCDC (Helm et al. 1996) and cv. Shyri from Ecuador (Vivar 2001). The two cultivars have been found to be consistently resistant to stripe rust in the screening nurseries (data not shown). Further work is needed to investigate the genetic resistance of the FCDC barley lines as being identified as resistant phenotypes using the international nursery data reported in the present paper.

ACKNOWLEDGEMENTS

We thank L. Hoge for managing barley germplasm and Zhiqiu Hu for input in statistical analysis. The funds provided by Alberta Crop Industry Development Fund to conduct the current research are gratefully acknowledged.

Brown, W. M., Hill, J. P. and Velasco, V. R. 2001. Importance of identifying stripe rust resistance in barley. Pages 18–23 *in* H. E. Vivar and A. McNab, eds. Breeding barley in the new millennium: Proceedings of an International Symposium. CIMMYT, Mexico, D.F.

Chen, X. M. 2007. Challenges and solutions for stripe rust control in the United States. Austr. J. Agric. Res. **58**: 648–655.

Fisher, R. A. 1925. Statistical methods for research workers. Oliver and Boyd, London, UK.

Hatcher, L. and Stepanski, E. J. 2005. A step-by-step approach to using the SAS system for univariate and multivariate statistics. 2nd ed. SAS Institute, Inc., Cary, NC.

Hayes, P. M., Castro, A., Corey, A. E., Filichkin, T., Rossi, C., Sandoval, J. S., Vales, I., Vivar, H. E. and Von Zitzewitz, J. 2001. Collaborative stripe rust resistance gene mapping and deployment efforts. Pages 47–60 *in* H. E. Vivar and A. McNab, eds. 2001. Breeding barley in the new millennium: Proceedings of an International Symposium. CIMMYT, Mexico, D.F.

Helm, J. H., Cortez, M. J., Jedel, P. E., Salmon, D. F. and Stewart, W. M. 1996. Registration of 'Seebe' barley. Crop Sci. **36**: 808.

Helm, J. H. 2001. Hulless barley: An overview. Pages 7–9 *in* H. E. Vivar and A. McNab, eds. Breeding barley in the new millennium: Proceedings of an International Symposium. CIMMYT, Mexico, D.F.

Kumar, K., Holtz, M. D., Xi, K. and Turkington, T. K. 2012. Virulence of *Puccinia striiformis* on wheat and barley in central Alberta. Can. J. Plant Pathol. doi: 10.1080/07060661.2012.722130.

Line, R. F. 2002. Stripe rust of wheat and barley in North America: A retrospective historical review. Annu. Rev. Phytopathol. **40**: 75–118.

Line, R. F. and Qayoum, A. 1991. Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America, 1968–87. U.S. Dep. Agric. Tech. Bull. No. 1788.

Littell, R. C., Stroup, W. W. and Freund, R. J. 2002. SAS for linear models. 4th ed. SAS Institute, Inc., Cary, NC.

Madden, L. and Pennypacker, S. P. 1979. Principal component analysis of tomato early blight epidemics. Phytopath. Z. **95**: 364–369.

McCallum, B. D., Fetch, T., Seto-Goh, P., Xi, K. and Turkington, T. K. 2006. Stripe rust of wheat and barley in Manitoba, Saskatchewan, and Alberta in 2005. Can. Plant Dis. Surv. **86**: 50.

McCallum, B. D., Pearse, P. G., Fetch, Jr., T., Gaudet, D., Tekauz, A., Seto-Goh, P., Xi, K. and Turkington, T. K. 2007. Stripe rust of wheat and barley in Manitoba, Saskatchewan and Alberta in 2006. Can. Plant Dis. Surv. **87**: 66.

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

Poland, J. A. and Nelson, R. J. 2011. In the eye of beholder: the effect of rater variability and different rating scales on QTL mapping. Phytopathology **101**: 290–298.

Puchalski, B. and Gaudet, D. A. 2011. Southern Alberta stripe rust survey. Can. Plant Dis. Surv. **91**: 69–70.

Roelfs, A. P. 1988. Genetic control of phenotypes in wheat stem rust. Ann. Rev. Phytopathol. **26**: 351–367.

Roelfs, A. P., Singh, R. P. and Saari, E. E. 1992. Rust diseases of wheat. Concepts and methods of disease management. CIMMYT, Mexico, D.F.

Rossi, C., Cuesta-Marcos, A., Vales, I., Gomez-Pando, L., Orjeda, G., Wise, R., Sato, K., Hori, K., Capettini, F., Vivar, H., Chen, X. and Hayes, P. 2006. Mapping multiple disease resistance genes using a barley mapping population evaluated in Peru, Mexico, and the USA. Mol. Breed. **18**: 355–366.

SAS Institute, Inc. 2010. SAS/STAT 9.2 user's guide. SAS Institute, Inc., Cary, NC.

Seem, R. C. and Gilpatrick, J. D. 1980. Incidence and severity relationships of secondary infection of powdery mildew on apple. Phytopathology **70**: 851–854.

Stevens, J. 1986. Applied multivariate statistics for the social sciences. Lawrence Erlbaum Associates, Inc., Publishers. Hillsdale, NJ.

Stuthman, D. D., Leonard, K. J. and Miller-Garvin, J. 2007. Breeding crops for durable resistance to disease. Adv. Agron. **95**: 319–367.

Vivar, H. E. 2001. Two decades of barley breeding. Pages 77–82 *in* H. E. Vivar and A. McNab, eds. 2001. Breeding

barley in the new millennium: Proceedings of an International Symposium. CIMMYT, Mexico, D.F.
Yan, G. and Chen, X. 2008. Identification of a quantitative trait locus for high-temperature adult-plant resistance against *Puccinia striiformis* f. sp. *hordei* in 'Bancroft' barley. Phytopathology **98**: 120–127.
Yang, R. C., Dhir, N. K., Yeh, F. C. and Hiratsuka, Y. 1997. Geographic variation in susceptibility of Alberta lodgepole pine to western gall rust. Can. J. For. Res. **27**: 1398–1405.
Zadoks, J. C., Chang, T. T. and Konzak, C. F. 1974. A decimal code for the growth stages of cereals. Weed Res. **14**: 415–421.