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Phenotypic Characterization of Canadian Barley Advanced Breeding Lines for Multiple Disease Resistance

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Fungal diseases pose a great challenge to Canadian barley production, among which are Fusarium head blight (FHB), yellow rust and scald. An integrated management approach is needed to mitigate these diseases, in which breeding for host resistance is the most effective component. Constant evaluation of advanced breeding lines for their resistance to the diseases is important for making steady progression. The main objective of this study was to screen 1,174 barley accessions, from a collaborative project between the Field Crop Development Center (FCDC), Alberta, Canada, and the International Maize and Wheat Improvement Center (CIMMYT), Mexico for their reactions to the three diseases. For FHB a 1–5 scale was employed to discard the very susceptible material in 2012 and 2013. In 2014, 514 most resistant lines having the score 1 in 2013 were re-evaluated in a replicated experiment. The most promising 166 genotypes were selected and advanced for their last evaluation in 2015 where FHB index was measured. Simultaneously, these 166 genotypes were subjected to two more experiments to test their reactions against stripe rust and scald. Eighteen two-rowed barley genotypes exhibiting broad-spectrum resistance to all of the three evaluated diseases were identified in addition to 40 lines combining FHB resistance with resistance to Mexican isolates and natural fungal population of either of the two foliar diseases and could be utilized in breeding programs aimed at improving resistance to multiple barley leaf and head blight diseases.

Keywords: Fusarium head blight, scald, stripe rust, multiple disease resistance

Introduction

Barley (*Hordeum vulgare* L.) is a major cereal crop in Canada, the European Union, Australia and many other countries. The average barley production in Canada during the period 2011–2014 was 8.28 million tons ranking 4th after Russia, France, and Germany. Western Canadian provinces including Saskatchewan and Alberta are the main barley producing provinces.

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Fusarium head blight (FHB) is a widespread devastating disease of barley and other small grains caused by various *Fusarium* species, mainly by isolates belonging to *F. graminearum* species complex (FGSC). In the past, FHB was a relatively infrequent and unimportant disease of barley in North America. It has emerged, in 1990s as a significant challenge reducing the yield and quality of barley in the US and Canada (Windels 2000; Legge et al. 2004), as is the situation in many other countries (Choo 2006). The increased prevalence of FHB was attributed by Tekauz et al. (2000) and Windels (2000) to the broad adoption of conservation agriculture, a possible shift in the pathogen populations toward aggressiveness, changes in rainfall patterns in addition to the widespread cultivation of commercial susceptible genotypes. Extensive collaborations of CIMMYT with research organizations in both developed and developing countries resulted in identification and continuous incorporation of new FHB resistance loci into elite CIMMYT germplasm. Screening strategy for identification of novel FHB resistant genotypes in wheat and barley in CIMMYT includes primary screening of 2,000+ entries in the first 2 years to discard the very susceptible lines which is normally done without replications due to the large number of entries to be evaluated and special attention is given to ensure genetic diversity in the selected materials. Only the most promising lines with high genetic diversity are advanced for later replicated experiments (He et al. 2013a; He et al. 2015; Osman et al. 2015b). This strategy of multi-year trial also prevents the escape of certain genotype under various environments (Miedaner et al. 2001), as evidenced in the above mentioned studies that lines showing very low FHB in the previous year may have a wide range of FHB severities in subsequent years.

The causal pathogen of barley stripe rust, *Puccinia striiformis* West., is associated with barley in most barley-growing areas leading to yield losses up to 100% in an epidemic (Line 2002; Chen 2005). The disease was not observed before 1987 in Mexico, nor was it significant before 1991 in the USA (Line 2002; Chen 2005). According to Dubin and Stubbs (1986), *P. striiformis* was introduced from Europe to America in the 1970s. Subsequently, the pathogen spread rapidly and established firmly in different locations of the USA.

Another severe disease, barley scald is a leaf and stem disease caused by the fungus *Rhynchosporium commune* (formerly *R. secalis;* Zaffarano et al. 2011). This disease frequently affects barley in the humid parts of the barley-growing areas and has the potential to develop rapidly under cool and wet conditions. Both grain yield and quality are detrimentally affected upon scald infection since grain weight is reduced in susceptible genotypes (Brown et al. 1996; Aoki et al. 2011). *R. commune* is a highly variable fungal pathogen (Goodwin et al. 1993; Zaffarano et al. 2006) with many pathotypes (Zhan et al. 2012) attributable to the large population sizes (Zhan et al. 2008). This high variability gives it an advantage to quickly overcome the resistance of individual genes in barley in a relatively short period (Zhan et al. 2008). To effectively control barley scald, continuous efforts on identification of novel genes and quantitative trait loci (QTLs) are therefore vital to keep pace with pathogen development (Wang et al. 2014).

The objectives of this study were to screen 1,174 barley accessions, from the collaborative project between the Field Crop Development Center (FCDC), Alberta, Canada, and

the International Maize and Wheat Improvement Center (CIMMYT), for their FHB resistance, and to test the reactions of the least FHB susceptible accessions to stripe rust and scald, in order to identify possible new parents for multiple disease resistance breeding.

Materials and Methods

A total of 1,174 advanced breeding lines at $F_{6:8}$ or later generations from the FCDC, Alberta, Canada, were used in this study. Chronologically the studied genotypes were compiled in two groups: Group1 included 552 advanced breeding lines that were sown in the summer season (May–September) and evaluated for FHB resistance from 2012 to 2015, while Group2 included 622 lines that were assessed from 2013 to 2015. Five genotypes with known FHB resistance were used as checks, including Seebe (resistant check), AC Metcalfe, CDC Copeland and AC Ranger [moderately resistant (MR) or moderately susceptible (MS) checks], and Kasota (susceptible check).

FHB field experiments were conducted at El Batan (altitude of 2,240 masl, latitude 19N, with an average annual precipitation of 625 mm) using the FHB sick plot. In 2012 and 2013, the experiments were done in 1 m double rows without replication, whereas in 2014 and 2015 lines were sown on May 14 and May 5, respectively, in a randomized complete block design with two replications. Checks were randomly distributed in the screening field. Field inoculation was conducted by spraying a suspension of a mixture of five highly aggressive and potent DON-producing isolates of *F. graminearum* that were collected from naturally infected Mexican fields and characterized as described previously (Osman et al. 2016). Barley spikes were spray inoculated at the heading stage when 50% of the spikes fully emerged in a plot and the inoculation was repeated twice at two days' interval. To enhance disease pressure in the nursery, barley/maize rotation and conservation agricultural practices were followed. An automated overhead mist irrigation system was used in the nursery following inoculation to supply adequate levels of moisture necessary for infection. The misting was set to operate automatically from 9 a.m. to 8 p.m., with 10 min of spraying per hour. FHB symptoms were investigated at 25 days post inoculation (dpi). In 2012 and 2013, FHB symptoms were evaluated on a whole plot basis using a 1–5 linear scale. Only the lines having the score 1 were advanced for evaluation in the subsequent year. In 2014 and 2015, ten random spikes of each plot (five per row) were tagged at heading by red sticky tape and were evaluated at 25 dpi. The numbers of total and infected kernels in each tagged spike were counted and used for estimating FHB index through the formula $FHB\ index\ (\%) = (Severity \times Incidence)/100$, where *Severity* stands for the averaged percentage of diseased kernels and *Incidence* for the proportion of symptomatic spikes.

Field screening of lines for scald was carried out four weeks after planting in the disease nursery at the Toluca experimental station, which is a cool and humid location with an average annual rainfall of 800 mm at an altitude of 2640 masl, latitude 19°N, in the State of Mexico, Mexico. Inoculation was carried out by spraying a spore suspension of *R. commune*, isolate CIMFU 1236 that was isolated and characterized from field block F6 in El Batan, Mexico. Spore concentration was adjusted at 1×10^5 spores/ml. Plants

were assessed at anthesis on whole plots using a 0–100 scale (0–10 = Resistant, 10–20 = Moderately Resistant, 20–30 = Moderately Susceptible, 40–50 = Susceptible and >50 = Very Susceptible). Seebe and CDC McGwire were used as resistant and susceptible checks, respectively.

For stripe rust, the plant material was also evaluated in 0.75 m double row plots at Toluca where weather conditions are conducive to regular natural stripe rust epidemics. The extremely susceptible barley lines 'Apigaco63' was planted two weeks earlier as spreader rows to initiate infection upon inoculating them with the stripe rust isolate 'Race 24' (Roelfs and Bushnell 1985), and spreader rows were spaced at 16-m intervals. 'Race 24' is of *P. striiformis* f. sp. *hordei*, which caused barley stripe rust epidemics globally and is still being used in many studies on barley stripe rust (Wan et al. 2017). Disease severity was scored on a plot basis using visual assessment of the percentage of crop canopy infected on a scale of 0–100%, where 0% for no symptoms and 100% for maximum symptoms when the majority of the genotypes were at GS 55 on the Zadock's scale. In this experiment, lines having disease severity 10% or less were considered to be resistant. Seebe and CDC McGwire were used as resistant and susceptible checks, respectively.

The field data were analyzed by R program (R Core Team 2015) ver. 3.0.2 for a randomized complete block design. Analysis of variance (ANOVA) was carried out with the *aov* command, and the ANOVA table components were used to estimate broad sense heritabilities (Lu et al. 2013).

Results

FHB incidence was low in 2012, with 435 (73%) lines in Group1 having a score of 1 and only one line having a score of 4, the highest disease score found in that year. Among the checks, only the susceptible check Kasota had a score of 2, and all the other four had a score of 1. In 2013, 435 lines from Group1 with the score 1 in 2012 were re-evaluated, and the disease development was better than the previous year with the proportion of the scores 1 through 5 being 53, 29.6, 13.7, 2.6, and 0.7%, respectively. Simultaneously, 622 from Group2 were planted for their first evaluation in 2013. About 51% (317 lines) of the least FHB susceptible genotypes were selected from Group2 and advanced for further evaluation. The susceptible check Kasota and the moderately susceptible check AC Ranger showed a score of 3, while the check AC Metcalfe scored 2 and other checks scored 1 in 2013.

In 2014, 217 and 317 most resistant lines from Group1 and Group2 having the score 1 in 2013 were re-evaluated in a replicated experiment for their third and second-year evaluation, respectively. The FHB index range extended from 3.74 to 42.16%, with the resistant check Seebe and the susceptible check Kasota being 12.25 and 48.47%, respectively (Fig. 1a). Based on this cycle of evaluation, we eliminated 368 susceptible accessions from further screening.

In 2015, all inoculated barley cultivars (including checks) developed FHB symptoms following spray inoculation. Similar to 2014, genotypes differed significantly in their

symptoms based on FHB index in 2015 and showed continuous variation for FHB index (Table 1, Fig. 1a). The resistant check Seebe had FHB index as low as 11.2% whereas susceptible check Kasota had the highest FHB index of 51.2%. It is noteworthy that 64

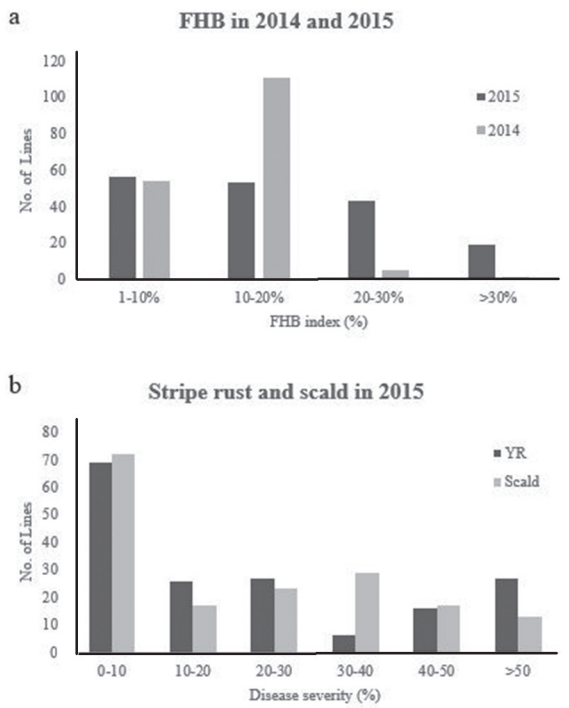

Figure 1. Frequency distribution of Fusarium head blight (FHB) in 2014 and 2015 (a), stripe rust and scald (b) in 2015

Table 1. Analysis of variance for Fusarium head blight index (FHB), Scald and Stripe rust in 2015

Trait	Source	DF	MS	*F* value	*P*
FHB	Genotype	169	156.53	2.07	<0.0001
	Rep	1	12.79	0.16	0.68
	Error	122	75.44		
Scald	Genotype	169	945.6	28.01	<0.0001
	Rep	1	506.5	15.00	<0.0001
	Error	169	33.8		
Stripe rust	Genotype	169	1040.7	3.43	<0.0001
	Rep	1	747.7	2.46	0.118
	Error	169	303.0		

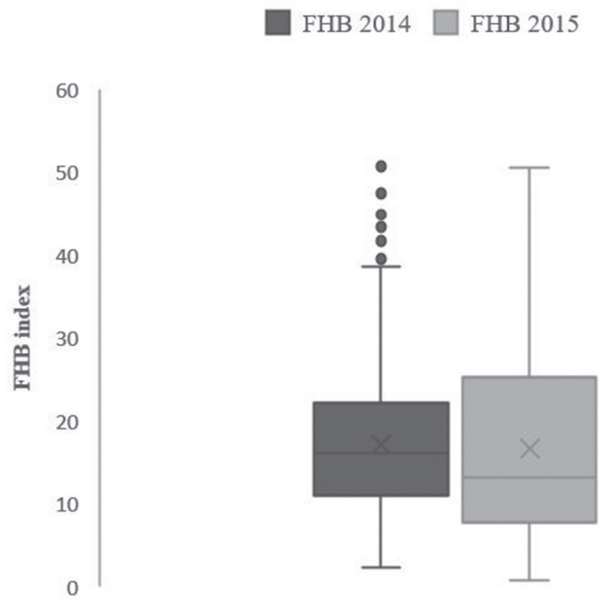

Figure 2. Boxplot of FHB index for barley genotypes grown in 2014 (n = 514) and 2015 (n = 166). The midline in each box represents the median, while the lower and upper whiskers represent 25 and 75 percentiles, respectively

lines had a FHB index less than the resistant check Seebe (Table S1*). The grand mean of FHB index in 2015 was slightly lower than in 2014, and the median value in 2015 is much lower than in 2014 (Fig. 2).

Evidently, six-rowed lines were more susceptible than two-row genotypes in both groups. This trend can be clearly seen from the large number of discarded six-rowed accessions following the first two cycles of screening. For example, 61 out of 72 lines scored 3 through 5 in 2013 from Group1 were six-rowed (data not shown) and it is noteworthy that only 22 six-row barley accessions were advanced for further testing in 2015.

The studied genotypes differed widely in their resistance to stripe rust. Disease severity ranged from 0 to 85%, with 69 resistant lines having disease severity less than or equals to 10% (Fig. 1b, Table S1). Similarly, 71 showed low levels of scald having severity less than or equals to 10% (Fig. 1b, Table S1).

Eighteen of the FHB resistant genotypes were also resistant to stripe rust and scald (Table 2). Another 40 FHB resistant lines also showed resistance to either stripe rust or scald, and 12 of the FHB susceptible lines were resistant to both stripe rust and scald. On the other hand, 27 genotypes were susceptible to all three studied diseases (Table S1).

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

Table 2. Barley genotypes evaluated in this study that exhibited resistance to all three diseases

Genotypes		FHB1	FHB2	YR	Scald
J02099003	10/2T0006	10	3	0	3
J05017005	13/3T0001	11	15	0	0
J04060004	11/2T0065	9	2	0	0
J06038160	13/2T0095	14	11	10	0
J06018021	13/2T0015	6	8	10	0
J02125002	11/3T0014	17	12	0	0
J02114001	10/2T0018	9	6	0	0
J05027002	12/2T0031	15	13	10	0
J06038132	13/2T0094	13	6	0	0
J06042111	13/2T0106	16	12	10	10
J04065003	11/2T0103	15	8	10	0
J06015157	13/2T0005	8	7	10	0
T07041002	12/3T0079	11	8	10	0
J06051005	13/2T0115	6	9	0	0
J04059004	12/3T0018	10	11	10	3
J04054003	12/3T0010	10	5	10	0
J04068002	13/4T0014	9	10	10	5
J04078005	11/2T0159	15	8	5	0
Resistant Check		11	11	0	8
Susceptible Check		51	53	85	65

FHB1 and *FHB2* stands for field FHB index (%) in 2014 and 2015, respectively; *YR* for stripe rust severity (%) in 2015, and *Scald* for scald severity (%) in 2015. The Canadian cultivar Seebe was used as resistant check for all three diseases, whereas Kasota was used as susceptible check for FHB and CDC McGwire for YR and scald. The complete dataset with disease data for all breeding lines could be accessed via https://www.dropbox.com/s/6boq5hd322bpqyc/00%20Raw%20data.xlsx?dl=0.

Discussion

Host resistance constitutes the most appropriate, effective, economical and environment-friendly approach to manage plant diseases, especially when other control measures are challenging. Due to the strict requirements in barley breeding for malting and brewing which are based on numerous quality traits (Wych and Rasmusson 1983), breeders are forced to cross closely related genotypes with known malting characteristics leading to a drastic decline in diversity. For example, Martin et al. (1991) reported that about 50% of the Minnesota barley germplasm parentage traces back to only five ancestors. Additionally, the scarcity of multiple resistant germplasm which combines disease resistance with desirable agronomic characteristics, e.g. early maturity, acceptable plant height, yield, and quality, highlights the importance of the continuous search for such genotypes taking

into consideration the maintenance of good levels of diversity. The present study was, therefore, taken up to identify putatively new multiple disease resistant barley lines. To this end, several independent field trials were conducted using artificial inoculation to evaluate the reactions of Canadian barley breeding lines to three important diseases including FHB, stripe rust and scald.

Contrary to wheat which has a broad variation for type II FHB resistance, barley has natural type II resistance because FHB symptoms usually do not spread within the rachis from initially infected spikelets to adjacent ones (Steffenson et al. 2003; Bai and Shaner 2004). Hence, differences in FHB resistance among barley genotypes should be mainly attributed to variation in type I resistance (Rudd et al. 2001; Steffenson et al. 2003; Bai and Shaner 2004). Additionally, type I resistance is more important from an epidemiological aspect since high levels of type I resistance are needed to prevent DON levels from exceeding the legal thresholds set by the malting and brewing manufacturers in malting barley cultivars. Hence, the development of barley genotypes with low FHB symptoms and low mycotoxin accumulation is the principal concern in barley breeding.

Spray inoculation of macroconidial suspensions on flowering heads is the main artificial inoculation method used in CIMMYT to evaluate FHB resistance since it reduces disease escape due to plant height because the inoculum is sprayed over the heads (He et al. 2013a; He et al. 2013b). The inoculum used for the spray inoculation trials consisted of a mixture of *F. graminearum* isolates in order to ensure an appropriate level of aggressiveness in variable environmental conditions. Furthermore, barley-maize rotation, as well as retention of crop residues, which serves as an additional source of inoculum are adopted in CIMMYT's FHB nursery to ensure adequate disease pressure sufficient to cover germplasm of wide-ranging maturity. Therefore, sowing date was planned so that the flowering encounter a big amount of precipitation to facilitate the rain-splash pathogen spread since a huge quantity of *Fusarium* inoculum is expected to be present on the soil surface. Although this procedure is necessary for encouraging natural infection, it is anticipated to affect the experiment reproducibility. Nevertheless, the FHB indices for the susceptible check Kasota were consistently high both in 2014 and 2015 indicating satisfactory levels of disease pressure. The grand mean of FHB index in 2015 was lower than in 2014, indicating the achieved progress in the selection process. Although the selection progress was not outstanding, it was still good due to the fact that disease pressure was higher in 2015 than in 2014 evidently from the number of lines having FHB index of 20% or higher (Fig. 1a), as well as the higher FHB index of the susceptible check Kasota in 2015 as was shown earlier.

Various morphological traits, e.g. plant height, days to heading, row-type, and hullessness have been found to show association with FHB resistance in barley (Choo 2006; He et al. 2015). The number of fertile florets for a rachis node, which is controlled by the *Vrs1* and *vrs1* loci for two-rowed and six-rowed barley, determines row-type (Komatsuda et al. 2007). Significant quantitative variation for FHB resistance was detectable among barley genotypes, and the majority of susceptible genotypes which were excluded following the first two cycles of evaluation were six-rowed. Thus, the general trend in our study that six-rowed barley tended to be more diseased than two-rowed barley is in agreement

with other reports (McCallum et al. 2004; Choo 2006; He et al. 2015). However, many six-rowed barley lines were selected and advanced to the final cycle in 2015 suggesting that it is still possible to find promising FHB resistant sources among six-rowed barley lines when adequate numbers of accessions are tested.

Up to now, 21 seedling resistance genes (*Rph1* to *Rph19*, *Rph21*, and *Rph22*) and two adult plant resistance genes (*Rph20* and *Rph23*) have been identified on all chromosomes except on chromosome 1H in *H. vulgare*. Compared with wheat rusts, barley stripe rust race structure and specificity are way less understood because only few studies on the molecular bases of the genetic resistance in barley. Although we used only one race in the artificial inoculation, a highly variable natural race complex prevalent in the nursery in Toluca region must have been involved due to the region being a hotspot for both diseases so the resistance is wider in this case as it would be by the single race.

Crop plants are simultaneously or sequentially targeted by various pathogens under field conditions as a result of conductive environmental conditions. For example, all of the studied diseases can be present in the same geographic location and season (Steffenson and Smith 2006; Guo et al. 2008; Kumar et al. 2012). However, the amount of primary inoculum and the resistance of grown genotypes affect disease pressure. Hence, breeding for multiple disease resistance is particularly advantageous and guarantees stability in barley production. Continued progress toward multiple disease resistance requires efficient phenotypic screening, to identify an adequate number of diverse resistant genotypes with desirable gene complexes assembled over decades of breeding favorable for acceptable malting characteristics and regional adaptation. Recently published reports are scarce on barley disease screening for a considerable number of accessions. CIMMYT adopted a unique shuttle testing strategy in which segregating lines are tested for different diseases in different locations in Mexico contrasting in latitude, altitude, and precipitation (Dubin and Rajaram 1996). Additionally, CIMMYT established a series of sick plot screening nurseries (e.g. FHB Screening Nurseries, FHBSN), which are well established and provide consistent and accurate disease reactions (He et al. 2013b; Osman et al. 2015b). Therefore, the present study aimed to identify multiple disease resistant barley under Mexican environments. Accessions that show resistance to two or more diseases are considered as multiple disease resistant. Combined resistance to FHB, scald and stripe rust was found in eighteen accessions wherein the most promising ones include J02099003 10/2T0006, J05017005 13/3T0001, J04060004 11/2T0065, J06038160 13/2T0095 and J06018021 13/2T0015. By strategically selecting accessions with broad resistance, multiple disease resistance can be incorporated into the barley breeding lines using single crosses.

As in our previous study on wheat (Osman et al. 2015a), it is interesting to note that a relatively small proportion of accessions exhibited resistance to all three diseases tested. Similarly, only a few accessions showed a high level of susceptibility to all of the evaluated diseases with the remainder of the accessions falling in between having resistance to either one or two diseases. Various resistance loci are expected to be present in the selected lines based on the number of tested genotypes and their pedigrees. However, further genetic analysis is needed to prove whether the resistances of these genotypes are

based on novel genetic makeup and whether their resistance is based on a gene/QTL cluster with broad spectrum effect on multiple pathogens or individual genes corresponding independently to each pathogen.

In summary, this study identified several putatively novel resistant barley sources with a broad spectrum of resistance and sufficient genetic diversity and agronomic superiority, which can be readily introduced into barley breeding programs and crossed with elite germplasm. The characterized lines were deposited in CIMMYT gene bank and the future perspective is to characterize their genetic makeup.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at https://akademiai.com/loi/0806

Electronic Supplementary *Table S1*. Barley genotypes and their reactions to Fusarium head blight (FHB) in 2014 and 2015, Stripe rust and Scald in 2015, and the number of diseases to which a genotype showed resistance