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AGRONOMIC PERFORMANCE OF HARD RED WINTER WHEAT LINES INTROGRESSED WITH THE *Fhb1* GENE

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Fusarium head blight (FHB) of wheat (*Triticum aestivum* L.), often results in significant economic losses to producers and end-users of the crop throughout the Great Plains of the United States. The development and release of resistant cultivars is an important component of an overall disease management strategy to minimize these losses, and perhaps the most cost-effective component. Spring wheat cultivars with *Fhb1*, a single gene resistance deriving from a Chinese source have been highly effective in limiting losses to the disease. However, as the source of resistance is not adapted to the Great Plains, there is a concern that cultivars with *Fhb1* also express lower grain yield. To address this concern for winter wheat, current study was conducted to evaluate 21 'Wesley' BC_2F_6 lines with *Fhb1* together with four adapted winter wheat check cultivars ('Arapahoe', 'Lyman', 'Overland', 'Wesley') without *Fhb1*for grain yield, grain volume weight, anthesis date, plant height, spikes per square meter, kernels per spike and thousand grain weight at two environments each in Nebraska and South Dakota. Trials at locations were planted to three replicates in an alpha-lattice design. Seven BC_2F_6 lines showed similar performance to Wesley for most of the measured traits including grain yield. One or more of those selected resistant lines may be released as cultivars with resistance to FHB or as parent germplasm for the development of high yielding FHB resistant winter wheat cultivars.

Keywords: Bread wheat, backcross breeding, fusarium head blight (FHB), winter wheat performance, FHB resistance

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is cultivated on about 240 million ha worldwide (Enghiad *et al*., 2017) and is a staple food crop for 40% of the world's population (Pandey *et al*., 2019) and it is estimated that about 21.5% of the crop's production is lost to disease or insect pests (Savary *et al*., 2019). US winter wheat producers of the Great Plains must annually contend with the potential economic losses caused by Fusarium head Blight (FHB). The most common causing pathogen of FHB is *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch] (Goswami and Kistler, 2004; Hernandez-Nopsa *et al*., 2014; McCartney *et al*., 2016). Grain yield and end-use quality losses resulting from infected plants include sterility of the florets, formation of discolored and shrunken kernels ("tombstone" kernels), and low grain volume weight (Tuite *et al*., 1990; McMullen *et al*., 1997; Dexter and Nowicki, 2003; Panthi *et al*., 2014). Diseased kernels may also contain significant levels of mycotoxins (primarily deoxynivalenol) that are hazardous to animal and human health, thus making the grain unfit for food and feed (McMullen *et al*., 1997; Bai *et al*., 2001; Sallam *et al*., 2017). Nganje *et al*. (2001) reported direct and secondary economic losses due to FHB for hard red spring (*T. aestivum*), soft red winter and durum wheat (*T. durum* L.), and barley (*Hordeum vulgare* L.) in the Northern Great Plains and Central United States at an estimated loss of $2.7 billion from 1998 to 2000 alone.

North American epidemics of FHB have periodically caused significant losses in wheat and barley throughout the 20th century (Leonard and Bushnell, 2003), and now, FHB is recognized as a disease of economic significance worldwide (Steiner *et al*., 2017). In the mid-1990s, there were severe FHB epidemics of varying intensities in the US (Windels, 2000). In Nebraska, FHB was first reported in 1898. Climate in Nebraska often favors FHB epidemics especially in eastern Nebraska where higher rainfall occurs during wheat flowering stage and early grain filling stage. In the past, major FHB epidemic years included 1957 and 1982, with the most resent FHB epidemic occurring in 2007 (http://www.ianrpubs.unl.edu/epublic/live/ec1896/build/ec1896.pdf, verified Mar. 14, 2018). The pathogen survives on crop residues (Haran *et al*., 2010) and soil, so there is always the potential for an FHB outbreak in the state when rain occurs at anthesis and early grain filling stage.

Several strategies have been described to alleviate losses caused by FHB (Wegulo *et al*., 2015). These strategies include cultural practices, chemical and biological control, planting resistant or tolerant cultivars, crop rotation, disease monitoring and improving harvesting strategies such as separating diseased kernels through combine harvester or seed cleaners. Most of these practices are only partially effective in controlling FHB (Buerstmayr *et al*., 2009). However, FHB resistant cultivars provide a cost-effective and environmentally safe alternative to FHB control (Steiner *et al*., 2017). Breeding for FHB resistant germplasm has become one of the main objectives in most of US wheat improvement programs.

Resistance to FHB is controlled quantitatively and progress to develop resistant cultivars has been slow worldwide (Wegulo *et al*., 2015). Wheat genotypes with tolerance to FHB resistance have been identified but many of the best adapted and high yielding cultivars are susceptible to FHB. The most effective gene, *Fhb1*, for FHB resistance is located on chromosome 3BS of Sumai-3 (*syn. Qfhs.ndsu-3BS*), which is a spring wheat cultivar developed in China (Cuthbert *et al*., 2006). However, winter wheat cultivars grown in the Great Plains do not carry this gene.

Efforts to develop small grains cultivars with enhanced resistance to FHB have increased by using traditional and molecular breeding approaches (Wegulo *et al*., 2015). The introgression of one or more target genes from a donor to the background of an elite cultivar or adapted germplasm is usually accomplished by backcrossing strategy (Semagn *et al*., 2006). The use of DNA markers during backcrossing greatly reduces the effort needed to backcross a disease resistance allele from a donor parent into a recurrent parent (Collard and Mackill, 2008). Cao *et al*. (2009) reported that marker-assisted selection was more effective than the conventional visual selection for improvement of resistance to FHB in a wheat backcrossing breeding program.

Jacobsen and Schouten, (2007) and Kang *et al*. (2011) reported that a QTL allele from an exotic parental source may be linked to deleterious genes and these deleterious genes may transmit together with the targ*et* allele when it is introgressed into a commercially adopted high yielding germplasm, which is also known as linkage drag. Previously Bakhsh *et al*. (2013), found that *Fhb1* did not have measurable negative effects on the agronomic and quality traits of hard red winter wheat.

The objective of the study was to determine the yield potential of selected Wesley backcrossing-derived *Fhb1* lines in the absence of FHB disease and identify best lines that carry *Fhb1* and have high yield potential to be released either as a new cultivar or as germplasm for parents to be used in future breeding.

MATERIALS AND METHODS

***Development of experimental material*:** A hard-red-winter wheat BC_2F_6 population of "WesleyFhb1" was developed collaboratively by the USDA-ARS Central Small Grain Genotyping Laboratory at Manhattan, KS, and the Department of Agronomy and Horticulture at the University of Nebraska Lincoln. The population was created by crossing an FHB resistant hard red spring wheat line ND2928 (North Dakota experimental line) carrying *Fhb1* with the winter wheat cultivar Wesley. Wesley was released in 1998 because of its high yield potential, superior bread-making quality and good adaptation in the north central Great Plains (Peterson *et al*., 2001). BC_1F_1 seed from the initial cross was sent to the United States Department of Agriculture (USDA) Genotyping Laboratory for additional development that followed a marker-assisted backcrossing strategy accompanied by phenotypic selection of backcross progeny for resistance to FHB (Fig. 1).

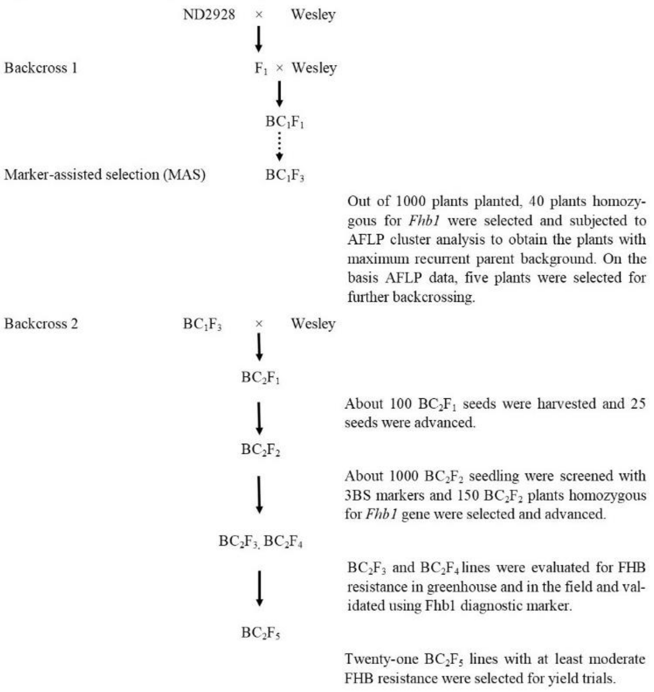

Figure 1. Schematic diagram of Wesley FhB1 BC population.

At the BC_2F_5, selected lines from the USDA were planted in Nebraska field nursery to obtain sufficient seed for grain yield and agronomic trait evaluations. A total of 21 BC_2F_6 WesleyFhb$_1$ lines were selected to have both *Fhb1* gene and a high level of FHB resistance. Those selected *Fhb1* lines and four check cultivars ('Arapahoe', 'Lyman', 'Overland' and Wesley) were planted for the 2010-11 growing season to 4 environments (Lincoln and Clay Center, NE; and Brookings and Dakota Lakes, SD). In each environment, the 25-entry experiment was comprised of three blocks (i.e., replications) arranged according to an alpha-lattice incomplete block design having five incomplete blocks of five entries each. Experiments in each environment were planted into conventionally tilled rain-fed fields. Recommended cultural practices for the area were applied. The planting date for each environment was optimal according to regional recommendations. To eliminate the confounding effects of diseases, the experiment at Lincoln was sprayed with

PROSARO^{TM}, fungicide (Bayer Crop Science, Durham, NC, 27709) according to the instructions of manufacturer at the initiation of flowering. Fungicide was applied to primarily suppress foliar fungal diseases that are common in the Lincoln environment; where as it was not applied in the other environments because in those, foliar diseases were not present at sufficient levels to potentially impact experimental results.

***Measurement of agronomic traits and data analysis*:** Agronomic traits including grain yield (GYLD) and grain volume weight (GVWT) were quantified in all environments. Plant height (PHT), anthesis date (AD), number of spikes per square meter (SPSM), kernels per spike (KPS) and 1000 kernel weight (TKWT) were measured at Lincoln and Clay Center. AD was visually assessed and measured as that period (days) from January 1 to the point when 50% of the spikes in a plot had extruded anthers. PHT was defined as an average height (cm) from the soil surface to the tip of the spikes, excluding awns and was measured once the plant reached it maximum height (usually 10 days after flowering). At harvest, four-row yield plots were cut, and GYLD (kg ha^{-1}) was quantified using a combine weigh scale. A 200-ml sample of wheat grains at 12-13% moisture contents was taken with a volumetric scale (Seedburo Equipment Co. Chicago, IL) to measure GVWT (kg hL^{-1}). 1000 seeds were counted with an electronic seed counter Agriculex ESC-1 (Agriculex Inc., Guelph, Ontario) and measuring their weight was measured to determine TKWT (g). Ten random spikes from four rows of each plot were harvested and threshed to determine KPS; whereas SPSM was estimated by dividing plot grain yield by kernels-per-spike and multiplying by single-kernel-weight. PROC MIXED procedure of SAS version 9.2 (SAS Institute, Cary, NC) was used for the analysis of variance (ANOVA) of the data collected in each environment. Before conducting combined ANOVA, heterogeneity of variance of error mean square in each individual ANOVA was tested with the F_{max} calculation; a value < 6 was considered homogeneous (Tabachnick and Fidell, 2007).

RESULTS AND DISCUSSION

From the combined ANOVA significant differences were observed among environments for all traits except for KPS and TKWT (Table1). Significant differences among the genotypes were observed for all traits. Finally, the genotype × environment interaction was significant for all traits except for AD and TKWT. The genotype × environment interaction was mainly due to changes in magnitude rather than changes in rank order (i.e., cross-over interaction). Because of this observation, genotypic means averaged over environments were examined and discussed hereafter.

Genotypic means (Table 2) were compared using a protected LSD ($P = 0.05$) test. First was made between the performance of Wesley, the recurrent parent, and the three widely grown check cultivars. Wesley, Lyman and Arapahoe had similar AD, which was significantly earlier than Overland. Wesley was significantly shorter than the other three check cultivars. Difference in GYLD was not significant among Overland, Wesley and Lyman, though Arapahoe had significantly lower yield than Overland. Difference in GVWT was not significant among Overland, Arapahoe and Wesley, but Lyman had significantly higher GVWT than Wesley. Overland had significantly higher SPSM than Arapahoe, but both were significantly higher than Lyman and Wesley. Wesley, Lyman, and Arapahoe had similar KPS, but KPS was significantly higher in Wesley than in Overland. Wesley had significantly higher TKWT than other three check cultivars.

To determine the relative performance of the WesleyFhb1 BC_2F_6 lines, the agronomic traits were compared between these BC_2F_6 lines and their recurrent parent, Wesley, using genotypic means over the environments. Of the 21 WeselyFhb1 lines, 18 had similar AD (ranging from 146.1 to 148.1 days) to recurrent parent Wesley. Two lines, WesleyBC58 and WeslyBC43, were significantly earlier than Wesley. These results are in agreement with Von der Ohe *et al*. (2010). Five WesleyFhb1 lines had similar PHT values (83.1 cm to 88.0 cm) to Wesley and remaining 16 lines were significantly taller than Wesley. The GYLD of 11 WesleyFhb1 lines (3661 kg ha^{-1} to 3817 kg ha^{-1}) were not

Table 1. Mean squares of traits measured for WesleyFhb1 population at 2 to 4 environments in Nebraska and South Dakota.

Source	DF	GYLD	GVWT	DF	AD	PHT	SPSM	KPS	TKWT
Environment	3	87896017**	185.66*	1	220.83**	12635.00**	152093.00**	276.16^{NS}	1.73^{NS}
Block (Env)	8	126320^{NS}	33.75**	4	6.47*	3.57^{NS}	1159.94^{NS}	38.45^{NS}	9.29^{NS}
Iblock (Env*Block)	48	158635**	4.04**	24	1.20^{NS}	27.67*	2740.28*	15.89*	7.77^{NS}
Entry	24	523874**	15.45**	24	6.94**	138.19**	16136.00**	34.27**	29.84*
Env*Entry	72	217136**	3.63**	24	1.41^{NS}	36.85**	4558.22**	21.34**	9.65^{NS}
Residual	144	92172	2.25	72	0.97	16.08	1364.86	9.51	6.97

GYLD = grain yield, GVWT = grain volume weight, AD = anthesis date, PHT = plant height, SPSM = spikes per square meter, KPS = kernels per spike and TKWT=1000-kernel weight, *Significance at the 0.05 probability level, ** Significance at the 0.01 probability level, ^{NS} Not significant at the 0.05 significance level

Table 2. Genotypic means of WesleyFhb1 lines over environments in Nebraska and South Dakota.

WesleyFhb1 Lines	AD (days)	PHT (cm)	GYLD (kg ha^{-1})	GVWT (kg hL^{-1})	SPSM	KPS	TKWT (g)
Arapahoe	146.7^{+}	102.8*	3815^{+}	72.53^{+}	357.4*	30.6^{+}	29.18*
Lyman	147.1^{+}	99.2*	4050^{+}	74.01*	298.4^{+}	30.8^{+}	32.50*
Overland	148.7*	98.5*	4483^{+}	73.51^{+}	451.0*	30.2*	30.26*
Wesley	147.2	85.6	4105	71.86	272.1	34.2	36.61
WesleyBC6	146.6^{+}	93.3*	3759^{+}	71.85^{+}	265.3^{+}	34.1^{+}	33.09*
WesleyBC10	148.0^{+}	99.7*	3615*	72.54^{+}	253.9^{+}	31.1^{+}	35.75^{+}
WesleyBC12	146.8^{+}	92.8*	3734^{+}	70.38^{+}	205.7*	40.0*	34.29^{+}
WesleyBC13	146.8^{+}	88.0^{+}	3518*	69.92*	230.0^{+}	35.4^{+}	34.85^{+}
WesleyBC19	147.5^{+}	92.5*	3691^{+}	71.73^{+}	217.1*	34.6^{+}	33.56^{+}
WesleyBC21	147.7^{+}	84.3^{+}	3388*	71.38^{+}	220.0*	35.0^{+}	33.08*
WesleyBC23	147.5^{+}	92.3*	3594*	72.58^{+}	235.3^{+}	34.7^{+}	33.67^{+}
WesleyBC32	147.2^{+}	83.1^{+}	3690^{+}	70.92^{+}	176.6*	37.3^{+}	37.68^{+}
WesleyBC39	146.1^{+}	91.7*	3740^{+}	71.74^{+}	232.5^{+}	35.3^{+}	33.48*
WesleyBC40	146.8^{+}	93.6*	3817^{+}	73.47^{+}	227.0*	32.8^{+}	38.68^{+}
WesleyBC41	147.3^{+}	95.8*	3631*	69.80*	245.0^{+}	33.6^{+}	35.83^{+}
WesleyBC43	143.4*	91.9*	3742^{+}	72.97^{+}	277.0^{+}	28.3*	36.48^{+}
WesleyBC46	148.1^{+}	94.8*	3508*	72.10^{+}	197.3*	34.9^{+}	36.04^{+}
WesleyBC47	148.8*	87.6^{+}	3661^{+}	70.15^{+}	267.4^{+}	34.4^{+}	31.12*
WesleyBC52	147.3^{+}	95.1*	3481*	69.52*	196.2*	35.6^{+}	36.81^{+}
WesleyBC56	147.5^{+}	93.0*	3604*	73.58^{+}	228.2^{+}	30.8^{+}	36.66^{+}
WesleyBC58	145.7*	91.8*	3661^{+}	70.97^{+}	232.0^{+}	34.0^{+}	34.98^{+}
WesleyBC59	149.1*	101.9*	3398*	71.90^{+}	201.5*	36.4^{+}	33.42*
WesleyBC88	146.7^{+}	91.9*	3679^{+}	71.97^{+}	216.1*	34.0^{+}	38.17^{+}
WesleyBC95	147.0^{+}	98.3*	3421*	71.05^{+}	196.9*	35.5^{+}	37.70^{+}
WesleyBC107	147.1^{+}	86.8^{+}	3713^{+}	70.95^{+}	250.2^{+}	34.2^{+}	33.77^{+}
Overall Mean	147.1	93.1	3700	71.73	246.0	33.9	34.70
Mean of WesleyFhb1 lines	147.1	92.4	3621	71.50	227.2	34.4	35.20
Range of WesleyFhb1 lines	143.4 to 149.1	83.1 to 101.9	3388 to 3817	69.52 to 73.58	176.6 to 277.0	28.3 to 40.0	31.12 to 38.68
LSD (0.05)	0.88	1.73	562.74	0.99	16.24	1.39	1.1
CV	1.19	12.20	28.24	3.75	31.06	13.02	9.99

AD = anthesis date, PHT = plant height, GYLD = grain yield, GVWT = grain volume weight, SPSM = spikes per square meter, KPS = kernels per spike, TKWT = 1000-kernel weight, + Similar to Wesley, * significantly different (higher or lower) than Wesley ($P < 0.05$)

significantly different from Wesley (4105 kg ha^{-1}), however none of the BC lines had higher GYLD than Wesley. Ten lines had significantly lower yield, suggesting additional backcrosses might be beneficial. A total of 18 WesleyFhb1 lines (70.15 kg hL^{-1} to 73.57 kg hL^{-1}) had similar GVWT to Wesley (71.85 kg hL^{-1}), and rest of three lines had significantly lower GVWT than Wesley.

A total of 12 WesleyFhb1 lines had similar SPMS (228 to 277) to Wesley (272) and the differences between *Fhb1* lines and the recurrent parent Wesley were not significant. KPS of WesleyBC12 line was significantly greater (40.0) than each of check cultivars and other WesleyFhb1 lines. A total of 19 WesleyFhb1 lines had KPS values from 30.8 to 37.3, which were not significantly different from Wesley. WesleyBC43 had significantly lower KPS than Wesley. For TKWT, 16 WesleyFhb1 lines were not significantly different (33.56 g to 38.67 g) from Wesley, but five lines had significantly lower TKWT than Wesley.

The objective of the study was to identify BC lines that carry *Fhb1* gene but had similar agronomic traits to widely adapted recurrent parent Wesley. We identified one line, WesleyBC107 that was similar to Wesley for all the traits measured in this study. Another line, WesleyBC40, was significantly taller and had fewer SPSM than Wesley but was similar to Wesley for the other traits. Two lines including WesleyBC6 and WesleyBC12 were similar to the recurrent parent Wesley for all traits except for being significantly taller and lower in TKWT. WesleyBC107, WesleyBC6, WesleyBC12 and WesleyBC40 can be considered as Fhb1 donor parents due to their higher GYLD. Similarly, other lines with higher GYLD like WesleyBC43, WesleyBC58 and WesleyBC39, which were earlier lines, might be good parents for these regions where earlier lines are needed.

In this study, we identified 11 lines that had similar values for the measured traits to Wesley, but no BC line with significantly higher grain yield than the recurrent parent Wesley was identified. Ten lines with significantly lower grain yield might be due to that on average BC_2 lines have 87.5% the recurrent parent genome and the 12.5% of ND2928 donor parent genome which may carry some undesirable QTLs for yield and adaptation. Using the molecular markers to select more Wesley alleles in the background or increasing the number of backcrosses would increase the percentage of recurrent parent genome in the BC lines and perhaps improve grain yield. We previously found that *Fhb1* did not significantly decrease yield (Bakhsh *et al*., 2013), and the purpose of backcrossing in currently study was to recover as much Wesley's genome as possible. Salameh *et al*. (2011) introgressed the *Fhb1* gene from Asian spring wheat by backcrossing into European winter wheat. Their BC_2 derived progenies were lower in yield but not significantly different from recurrent parent, which agreed with the result from the current study.

It should be noted that Wesley is semi-dwarf wheat cultivar and only five BC lines had similar plant height to Wesley. The remaining 16 lines were significantly taller than Wesley, which indicated that Wesley had multiple genes for plant height and those genes were not captured using the two backcrosses. It is also possible that some taller stature alleles may be linked to *Fhb1* (Fedak *et al*., 2008) because most of the lines were taller than Wesley. Wesley may have several short statured genes, and is one of the shortest semi-dwarf lines grown in Nebraska.

For grain yield and plant height, the BC lines had either similar to or lower yielding than Wesley, which suggests Wesley contributed most of beneficial alleles for these traits and in the backcrossing progeny. For the other traits measured in the BC lines, always some BC lines showed greater values than Wesley, indicating that ND2928 may also carry some beneficial genes for these traits.

Conclusion**:** We can conclude that some WeselyFhb1 lines that had similar or better agronomic characteristics, especially grain yield, can be used as parents to develop high-yielding and FHB-resistant cultivars. However, none of the selected WeselyFhb1 lines, can be recommended for cultivar release except in the areas where FHB is frequent and severe.

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