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Characterization of agronomic traits in a population of wheat derived from *Triticum timopheevii* and their association with Fusarium head blight

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Abstract Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, is one of the most destructive diseases of wheat especially in humid and semi-humid wheat growing areas of the world. Agronomic traits such as plant height, flowering date, and presence/absence of awns play important roles in wheat life cycle and grain yield. In the present study, characterization of agronomic traits and their association with Fusarium head blight were investigated in a population of recombinant inbred lines (RILs) derived from the cross of Canadian bread wheat cultivar 'AC Brio' to *Triticum timopheevii*-derived line 'TC 67'. The population was grown under both greenhouse (Winnipeg, MB, Canada) and field (Carman and Glenlea, MB, Canada) conditions and measurements were made for agronomic traits including plant height, flowering date, presence/absence of awns, and glume threshability. The wheat genotypes were also assessed in the greenhouse for disease severity (type II resistance) and in field plots for disease incidence (type I resistance), disease severity (indicating a combination of type I and type II resistance), deoxynivalenol (DON) content (type III resistance), and Fusarium-damaged kernels (FDK) which is an indicator of type IV resistance. Results showed that there was significant variation in plant height, flowering date, and FHB resistance among the genotypes, and the

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frequency distributions of these traits were continuous, indicating the quantitative inheritance of the traits. While both plant height and flowering date showed negative correlations with disease incidence, severity, and DON content, they both were positively correlated with FDK under field conditions. In the greenhouse, both plant height and flowering date were positively correlated with disease severity. Awnedness and seed threshability were also significantly associated to all FHB-related traits. While the presence of awns was associated with a significant decrease in FHB, the genotypes that were easy to thresh were more susceptible to the disease. The line 'TC 67' with novel FHB resistance derived from *T. timopheevii* may be useful in wheat breeding, but the positive association of disease resistance with undesirable agronomic traits such as plant height may delay cultivar development efforts.

Keywords *Triticum timopheevii* · AC Brio · TC 67 · Recombinant inbred lines · Agronomic traits · Fusarium head blight

Introduction

Agronomic traits such as plant height, flowering date, presence/absence of awns, and glume threshability play important roles in wheat life cycle and grain yield. Plant height is a major agronomic charasteristic in wheat because of its association with lodging and yield. In bread wheat, there is a negative correlation between plant height and yield (Law et al. 1973). During the past 50 years, the average height of bread wheat has decreased considerably while wheat yield has steadily increased. Flowering date is another important trait that plays a major role in regional and seasonal adaptation and attaining desired yield in wheat (Zhang et al. 2009). Even though grain yield generally is greater with late maturity than with early maturity, late-maturing lines can be adversely affected by biotic and abiotic stresses such as diseases, heat, drought, and end of season frost. Awns are another agronomic trait which may play a major role in photosynthesis and yield increase in wheat, especially under drought conditions (Evans et al. 1972). In addition, awns are ideally placed for light interception and CO_2 uptake, the pathway for assimilate movement from awns to the kernel being minimal (Evans et al. 1972). Moreover, they have less tendency to senesce than the flag leaves since they develop later (Sourdille et al. 2002). During domestication process of wheat, changes in spike morphology including rachis fragility and glume threshability enhanced its suitability for agricultural uses (Nalam et al. 2007). It has also been important in terms of resisatnce to FHB in wheat (Steiner et al. 2004). Based on rachis fragility and glume threshability, hexaploid wheat (*Triticum aestivum*, $2n=6\times=42$, AABBDD) has historically been divided into six subspecies: *vulgare* (common wheat), *sphaerococcum*, *compactum*, *spelta*, *macha*, and *vavilovii* (Kimber and Sears 1983). While the subspecies *sphaerococcum*, *compactum*, and *vulgare* have a non-brittle rachis with free-threshing glumes (Sears 1947), the subspecies *spelta*, *vavilovii*, and *macha* have a fragile rachis, and are not free-threshing (Kabarity 1966).

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch], is one of the most devastating diseases of wheat in many wheat growing regions worldwide. Resistance to FHB is highly complex due to its quantitative inheritance and the high genotype by environment interactions. Five types of genetic resistance to FHB have been described in wheat. They are: resistance to initial infection (type I), to fungal spread within plant tissues (type II) (Schroeder and Christensen 1963), to toxin accumulation (type III), to kernel infection (type IV), and tolerance (Miller et al. 1985; Wang and Miller 1988; Mesterházy 1995). From another point of view, resistance to FHB in wheat involves active and passive mechanisms (Mesterházy 1995). Active resistance involves a direct physiological or biochemical interaction between plant and pathogen, while passive resistance involves the reduction of disease in plants due to traits that are independent of the presence of the pathogen.

While FHB may be controlled using foliar fungicides, the best and most economical method of disease control is the use of resistant cultivars. However, breeding for resistance to FHB is difficult as the most resistant sources are of exotic origin with poor agronomic traits, the inheritance of resistance is complicated, and screening for FHB resistance is environmentally biased, labour-intensive, and costly (Buerstmayr et al. 2002). Clarification of the relationship between FHB resistance and agronomic traits is of great importance for development of resistant wheat cultivars with good agronomic characteristics. Various morphological and agronomic traits such as flowering date, plant height, head compactness, and flower opening have been shown to be associated with resistance to FHB in wheat. These traits may confer passive resistance (Mesterházy 1995) by increasing the probability of disease escape rather than increasing the host resistance as a result of genetic factors (Kolb et al. 2001). On the other hand, resistance to FHB is sometimes associated with undesirable agronomic characteristics (Bai and Shaner 2004).

Relatively few sources of FHB resistance are known in wheat. Most breeding programs have utilized the Sumai3 or Frontana sources, but accessions from wild species of wheat have also been identified with good FHB resistance (Bai and Shaner 2004). Recently, a wheat line derived from *Triticum timopheevii* Zhuk, 'TC 67', with a high level of FHB resistance has been described (Cao et al. 2009). Transfer of resistance genes from non-adapted wheat sources into cultivated wheat generally involves unpredictable effects on agronomic and quality performance (Dvorjak 2014). In the other words, the desired resistance traits may be confounded with 'wild' plant traits such as excessive height or spike morphology (Buerstmayer et al. 2011). The objective of the present study was to determine the association of plant height, flowering date, presence of awns, and glume threshability with FHB resistance in a population of recombinant inbred lines (RILs) derived from the cross of two wheat genotypes, 'AC Brio' and 'TC 67'.

Materials and methods

Plant materials

A population of 230 F_7 recombinant inbred lines developed from the cross of two hexaploid spring wheat, 'AC Brio' and 'TC 67', was used in this study. 'AC Brio' is a relatively short, early-maturing, awnless, free-threshing Canadian bread wheat cultivar with moderate susceptibility to FHB. The pedigree of 'AC Brio' is as follows: 'Columbus'/S68147//'Laval19'/'Columbus'. 'TC 67' is a *Triticum timopheevii*-derived, tall, late-maturing, awned, non-free-threshing line with high level of resistance to FHB. 'TC 67' was developed from a cross between the FHB-susceptible spring wheat cultivar 'Crocus' (*T. aestivum*, $2n=6\times=42$, AABBDD) and a FHB-resistant accession of *T. timopheevii* ($2n=4\times=28$, A^tA^tGG), with the resulting F_1 backcrossed to 'Crocus' (Cao et al. 2009).

Greenhouse experiments

The RILs population and the parents were evaluated for plant height, flowering date, and disease severity in the greenhouse in 2007 in Winnipeg, MB. The experimental layout was a randomized complete block design with three replicates. An experimental unit consisted of three plants growing in a $16\times13\times13\text{-cm}^3$ pot. A mixture of four highly aggressive isolates of *F. graminearum* (M6-04-4, M9-04-6, M1-04-1, and M8-04-3) stored at the Cereal Research Centre (CRC) in Winnipeg was used for inoculum production and greenhouse inoculations. When spikes reached 50 % anthesis, they were inoculated by injecting 10 μl of conidial suspension (5×10^4 macroconidia/ml) into a floret positioned one-third distance from the top of the spike. At least five spikes in each pot (replication) were inoculated. Spikes were covered with $20\times5\ \text{cm}^2$ glassine bags (Seedburo Equipment Co., Chicago, IL, USA) for 48 h to maintain constant high humidity. The greenhouse had a photoperiod of 16 h light supplemented with incandescent high pressure sodium lights (OSRAM SYLVANIA LTD; Mississauga, ON, Canada). The temperature of the greenhouse averaged 24 °C during the day with a range of 18–28 °C, and 18 °C at night with a range of 16–20 °C.

Measurements were taken for agronomic and FHB-related traits. Plant height was measured as the distance from the soil surface to the top of the spike without awns at Zadoks growth stage 91 (Zadoks et al. 1974). Plants were scored for presence/absence of awns at the same time. Flowering date was determined as the average number of days from seeding date to anthesis for the first five spikes reaching anthesis in each pot. Disease severity (type II resistance) was scored as the percentage of diseased spikelets per spike at 21 days after inoculation.

Field experiments

The RILs population and the parents were evaluated for plant height, flowering date, presence/absence of awns, glume threshability, disease incidence, disease severity, deoxynivalenol (DON) content, and Fusarium-damaged kernels (FDK) under field conditions. Plots were established at two locations (Carman and Glenlea) in . The experimental designs in both locations were randomized complete blocks with three replicates.

Experimental units consisted of 1 m (Carman) or 1.5 m (Glenlea) length rows with 30 cm row spacing.

A mixture of the isolates 40/04, 71/04, 98/04, 136/04, MSDS 3/03, and EMMB 19/03 of *F. graminearum* was used for inoculations at Carman. The same mixture was used at Glenlea, except that the isolates M1-04-5 and M3-04-3 were used in lieu of MSDS 3/03 and EMMB 19/03. The inoculum concentration used in the field was the same as used in greenhouse inoculations. Plots were spray-inoculated individually at 50 % anthesis using a CO_2-powered backpack sprayer till runoff of the inoculum on the plants. Inoculation was repeated 2 or 3 days later. Mist (Carman) or sprinkler (Glenlea) irrigation was operated continuously for 1 h after inoculation to provide favorable conditions for fungal germination and penetration. At Carman, the mist system was applied at a rate of 5 min per hour for an additional 12 h. After the first day of inoculation, at both locations the irrigation system was operated 2–3 times a day, for 10–15 min each time, till the disease developed in the nurseries.

Data of agronomic traits including plant height, flowering date, and presence/absence of awns and FHB statistics including disease incidence and severity were collected during the growing season, while glume threshability, DON content, and FDK were determined post harvest. Plant height was measured as the distance from the soil surface to the top of the spikes minus awns, and flowering date as the number of days from seeding to 50 % anthesis of the plot, both at growth stage 91 according to Zadoks *et al.* (Zadoks et al. 1974). The genotypes were individually scored for disease incidence and severity 3 weeks after inoculation. Disease incidence (type I resistance) was determined as the percentage of diseased spikes per plot. Disease severity (combination of type I and type II resistance) was scored on a 0–100 % scale for infected spikelets on a whole-plot basis. Wheat rows were sickle-harvested at maturity and threshed using a Wintersteiger Nursery Master Elite combine (Wintersteiger AG, Ried, Austria). A wheat head thresher (Precision Machine Co. Inc, Lincoln, NE, USA) was later used as needed to thresh lines that had tightly-adhering glumes. Glume threshability was scored using a 1–3 scale (Wise et al. 2001) where: 1= free-threshing so that naked seeds dropped free of the glumes when spikes were crushed manually, 2=not free-threshing but glumes could be torn off with forceps to free the seed, and 3=not free-threshing and glumes could only be removed by scraping. DON accumulation (type III resistance) was measured using an ELISA method described by Sinha et al. (1995). FDK (type IV resistance) was assessed by counting the visually damaged kernels in three random sub-samples of 100 grains from each plot.

Statistical analysis

Statistical analyses were performed using SAS® 9.2 (SAS Institute Inc., Raleigh, NC, USA). Spearman correlation coefficients between agronomic and FHB-related traits were estimated using the PROC CORR procedure. Greenhouse and field data were tested for normality using the PROC UNIVARIATE procedure. If the residuals of the dependent variables did not follow a normal distribution, the appropriate transformations were applied. Analysis of variance (ANOVA) were performed using the PROC MIXED procedure. The effects in the statistical model for the greenhouse data analysis were genotype and block, and for the combined field data (two locations in 1 year) were genotype, location, genotype x location, and block. Genotype effect was considered fixed and the other effects were considered random. PROC TTEST was used to perform Student's *t* test to compare disease between two classes of awnedness and glume threshability, *i.e.*, with vs. without awns, and free-threshing vs. non-free-threshing.

Results

Agronomic traits

High variation in plant height and flowering date was observed among the genotypes under the greenhouse and field conditions, and the frequency distributions of both traits were continuous (Fig. 1). ANOVA using the greenhouse data or using the combined data of two locations under field conditions indicated significant differences among the genotypes for both traits (data not presented). Plant height in the greenhouse ranged from 72 to 130 cm with an overall mean of 102 cm for the population, and was 74 cm for 'AC Brio' and 130 cm for 'TC 67' (Fig. 1a). Under field conditions, plant height ranged from 76 to 118 cm with an overall mean of 102 cm for the population and 95 and 120 cm for 'AC Brio' and 'TC 67', respectively (Fig. 1b). Flowering date ranged from 56 to 87 days in the greenhouse with an overall mean of 70 days for the population, and was 63 days for 'AC Brio' and 74 days for 'TC

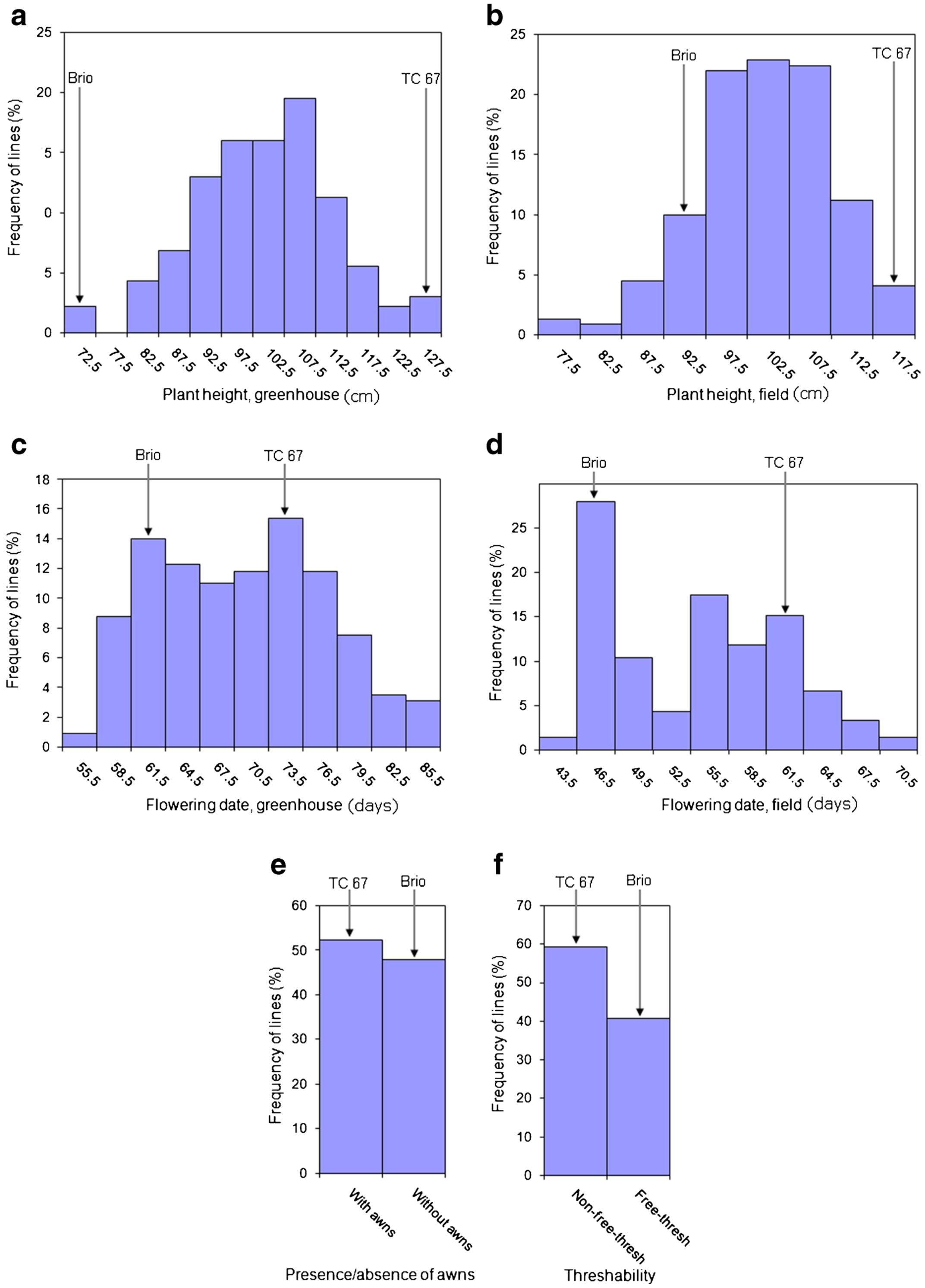

Fig. 1 Frequency distributions of agronomic traits among 230 recombinant inbred lines from the cross 'AC Brio'/'TC 67' under field and/or greenhouse conditions. Field data were based on the combined data of two locations, Carman and Glenlea, Manitoba in 2006, and the greenhouse data collected in Winnipeg, Manitoba, in 2007. Values of the parental lines are indicated by arrows

67' (Fig. 1c). Using the combined data of two locations under field conditions, a range of 45–70 days and an average of 55 days for the population were observed in flowerin date, with the values of 46 and 63 days for 'AC Brio' and 'TC 67', respectively (Fig. 1d).

In the present study, frequency distributions and mode of inheritance of other agronomic traits, *i.e.*, presence/absence of awns and glume threshability, were also investigated. There were 120 lines with awns, and 110 lines without awns (Fig. 1e). This fits a 1:1 ratio (χ^2=0.43, P>0. 5), indicating a single major gene is controlling this trait in the population. We found that 86 of the genotypes were free-threshing, and 125 were non-free-threshing (Fig. 1f), which does not fit a 1:1 ratio (χ^2=7.21, P<0.01).

Fusarium head blight resistance

High variation in disease severity was observed among the genotypes in the greenhouse, and the frequency distribution of the data was continuous (Fig. 2). ANOVA of disease severity data collected in the greenhouse showed significant differences among the genotypes (data not shown). Disease severity ranged from 5.2 to 92.4 % with an average of 35.3 % for the population, and was 35.3 % for 'AC Brio' and 5.4 % for 'TC 67' (Fig. 2a).

A high range of variation was also observed in all FHB-related traits including disease incidence, disease severity, DON content, and FDK under field conditions, and the frequency distributions of the traits studied in the population were continuous (Fig. 2). ANOVA of the combined data across locations indicated significant differences among the genotypes for all traits except FDK, and the effect of genotype x location was significant for all traits (data not shown). Disease incidence ranged from 4.9 to 87.7 % with an average of 23.9 % for the population and the values 31.7 % for 'AC Brio' and 7.7 % for 'TC 67' (Fig. 2b). A range of 9.7–70.0 % and an average of 33.4 % in disease severity were observed for the population and the values of 30.8 and 9.7 % for 'AC Brio' and 'TC 67', respectively (Fig. 2c). For DON content, a range of 0.0–4.4 ppm and an average of 1.5 ppm were observed in the population in the field and the values of 2.4 and 0.3 ppm for 'AC Brio' and 'TC 67', respectively (Fig. 2d). FDK ranged from 1.2 to 22.0 % with an average of 7.1 % for the population, and the values of 4.8 % for 'AC Brio' and 2.9 % for 'TC 67' (Fig. 2e).

Association of agronomic traits with Fusarium head blight

In general, agronomic traits were not highly correlated with FHB. Based on the results of greenhouse tests, both plant height and flowering date showed positive poor correlations with disease severity, of them the correlation between flowering date and the disease was significant (r^2=0.18, P<0.01). While both plant height and flowering date showed negative correlations with disease incidence, severity, and DON content under field conditions, they both had positive correlations with FDK, even though the correlation between plant height and FDK was not sighnificant (Table 1).

Analyses of the combined data sets from field experiments using Student's t test showed significant differences between genotypes with and without awns for all disease-related variables (Table 2). Results of the t test using single environment data sets from field experiments were also similar (data not shown). Lines with awns had significantly less FHB disease compared to lines without awns. Similar results were found in the greenhouse tests, where the presence of awns significantly decreased disease severity (Table 2).

The effect of glume threshability on FHB was also investigated. There were no class 3 lines detected for threshing habit (Wise et al. 2001), lines were either free-threshing or non-free-threshing. Analysis of the combined data sets from two locations showed significant differences between free-threshing and non-free-threshing genotypes for all FHB-related variables (Table 3). Lines that were non-free-threshing like the TC 67 parent, had significantly less FHB disease. Similar results were found for disease severity in the greenhouse, where genotypes that were difficult to thresh were more resistant to FHB (Table 3).

Discussion

High variation in plant height was observed among the genotypes of the RILs population with a continuous frequency distribution indicating the quantitative nature of the trait. Genotypes shorter than the short parent ('AC Brio') were observed in the population under both greenhouse and field conditions, which indicates transgressive segregation for plant height. Studies have indicated that plant height in wheat shows complex inheritance and is controlled by major and minor genes

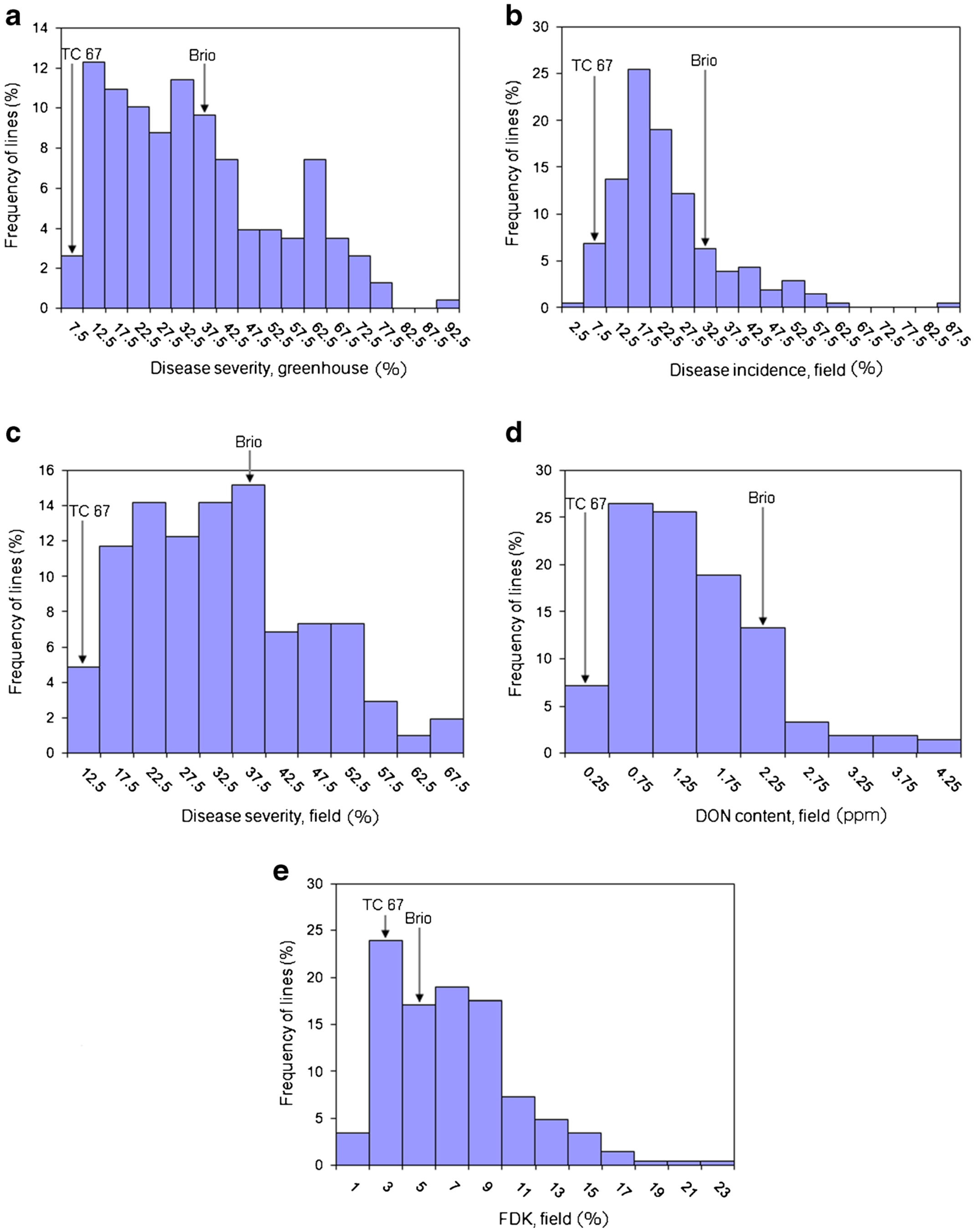

Fig. 2 Frequency distributions of FHB-related traits among 230 recombinant inbred lines from the cross 'AC Brio'/'TC 67' under field and/or greenhouse conditions. Field data were based on the combined data of two locations, Carman and Glenlea, Manitoba in 2006, and the greenhouse data collected in Winnipeg, Manitoba, in 2007. Values of the parental lines are indicated by arrows

(Worland et al. 2001). The catalogue of gene symbols in wheat (McIntosh et al. 1998) lists 21 genes of major effect with *Rht* symbols for plant height. Several studies also have detected QTLs for plant height (Bullrich et al. 2002; Gervais et al. 2003; Paillard et al. 2004; McCartney et al. 2005; Schmolke et al. 2005; Klahr et al. 2007). The RILs population of wheat derived from the cross of 'AC Brio' and 'TC 67' investigated in the

Table 1 Spearman correlation coefficients between agronomic traits and Fusarium head blight among 230 recombinant inbred lines from the cross 'AC Brio'/'TC 67' using field (F) and greenhouse (G) data[a]

Agronomic traits	FHB-related traits				
	D. incidence (F)	D. severity (F)	DON (F)	FDK (F)	D. severity (G)
Plant height (F)	−0.23**	−0.26**	−0.30**	0.11	-
Plant height (G)	-	-	-	-	0.04
Flowering date (F)	−0.24**	−0.41**	−0.39**	0.29**	-
Flowering date (G)	-	-	-	-	0.18**

[a] Field data (F) from two locations, Carman and Glenlea, Manitoba, in 2006, and the greenhouse data (G) based on data collected from the greenhouse in Winnipeg, Manitoba, in 2007

** Significant at $P<0.01$ probability level

present study was relatively tall with an overall mean of 102 cm. There is no record of reduced height (*Rht*) genes in either of the parents or any other ancestors of the population. However, there is a possibility of presence of *Rht* genes or QTLs for short straw at least in the susceptible parent ('AC Brio') with an average height of 74 cm.

Significant variation in flowering date was also observed among the genotypes under both greenhouse and field conditions, and the frequency distribution of the trait was continuous, indicating the quantitative nature of the trait. The average time to flowering for the population was relatively short: 70 days in the greenhouse and only 55 days under field conditions. This difference may be attributed to difference in environmental conditions such as temperature, humidity, and light. Transgressive segregants outside the range of both parents were found within the population in both environments. As the population and the parents under this study were spring wheat, in general, there is no possibility of vernalization (*Vrn*) genes affecting flowering date in the population. However, some spring wheat genotypes still have a short vernalization requirement and this can lead to delays in heading time. On the other hand, photoperiod requirement of the population was fulfilled during the plants' life cycle under experimental conditions in the greenhouse and field (at least 16 h of light during a day). Therefore, most probably, the flowering date was not affected by photoperiodism (*Ppd*) genes either. It is possible that earliness *per se* (*Eps*) genes or QTLs originating from either of the parents have been involved in flowering date. Numerous studies have reported the effects of *Eps* genes on flowering date in wheat (Law et al. 1976; Scarth and Law 1983; Hoogendorn 1985; Worland 1996; van Beem et al. 2005; Hanocq et al. 2007; Iqbal et al. 2007; Baga et al. 2009; Rousset

Table 2 Comparison of means of Fusarium head blight-related variables between two classes of recombinant inbred lines (without and with awns) from the cross 'AC Brio'/'TC 67' using field (F) or greenhouse (G) data sets[a]

FHB-related traits	presence/absence of awns	Number of genotypes	Mean values	Standard deviation	Standard error	t value	Pr>|t|
D. incidence (F):	Without awns	87	27.3	18.8	0.84	−6.53	<0.0001
	With awns	104	20.3	16.3	0.67		
D. severity (F):	Without awns	87	37.1	19.4	0.86	−5.43	<0.0001
	With awns	104	30.7	19.5	0.80		
DON (F):	Without awns	92	1.8	1.5	0.07	−6.30	<0.0001
	With awns	120	1.3	1.3	0.05		
FDK (F):	Without awns	87	8.3	8.1	0.36	−5.22	<0.0001
	With awns	104	6.0	6.6	0.27		
D. severity (G):	Without awns	95	39.2	23.2	1.36	−3.77	0.0002
	With awns	121	32.4	22.0	1.18		

[a] Field data (F) were the combined data of two locations, Carman and Glenlea, Manitoba, in 2006, and the greenhouse data (G) were collected from the greenhouse in Winnipeg, Manitoba, in 2007

Table 3 Comparison of means of Fusarium head blight-related variables between two classes of recombinant inbred lines (free-thresh and non-free-thresh) from the cross 'AC Brio'/'TC 67' using field (F) or greenhouse (G) data sets[a]

FHB-related traits	Glume threshability	Number of genotypes	Mean values	Standard deviation	Standard error	t value	Pr>|t|
D. incidence (F):	Free-threshing	79	26.3	18.2	0.86	4.16	<0.0001
	Non-free-threshing	117	21.7	17.2	0.68		
D. severity (F):	Free-threshing	79	36.1	19.1	0.90	3.48	0.0005
	Non-free-threshing	117	32.0	20.0	0.79		
DON (F):	Free-threshing	86	1.8	1.6	0.07	6.14	<0.0001
	Non-free-threshing	125	1.3	1.2	0.05		
FDK (F):	Free-threshing	79	8.7	7.8	0.36	6.60	<0.0001
	Non-free-threshing	117	5.8	6.6	0.26		
D. severity (G):	Free-threshing	85	48.0	23.3	1.46	9.19	<0.0001
	Non-free-threshing	125	30.8	19.1	0.99		

[a] Field data (F) were the combined data of two locations, Carman and Glenlea, Manitoba, in 2006, and the greenhouse data (G) were collected from the greenhouse in Winnipeg, Manitoba, in 2007

et al. 2011). Several QTLs have also been mapped on different chromosomes for heading date (Sourdille et al. 2000; Bullrich et al. 2002; McCartney et al. 2005; Heidari et al. 2012).

High variation was observed among the RILs for disease severity in the greenhouse, and for disease incidence, disease severity, DON content, and FDK under field conditions. In addition, the frequency distributions of all traits were continuous which is indicating quantitative inheritance of resistance to FHB. The RILs with values less than the resistant parent ('TC 67') or higher than the susceptible parent ('AC Brio') were observed among the population for all traits, indicating transgressive segregation.

Results of the present study showed that plant height was negatively correlated with FHB incidence, disease severity, and DON content under field conditions. In the other words, taller lines tended to be less diseased than shorter ones. Following the overall population, levels of the FHB-related traits in the taller parent 'TC 67' was lower compared to the shorter parent 'AC Brio'. A negative association between plant height and FHB has previously been reported by several authors (Hilton et al. 1999; Gervais et al. 2003; Paillard et al. 2004; Schmolke et al. 2005; Klahr et al. 2007). These observations support the hypothesis that shorter genotypes are more subject to infection by *Fusarium* due to higher moisture and humidity from the soil surface, thus enhancing disease development (Buerstmayr et al. 2000; Somers et al. 2003; Klahr et al. 2007). Additionally, spikes of taller plants may dry faster, leading to less FHB. These phenomena are categorized as a passive resistance mechanism of reducing disease in wheat. However, in the present study, we applied spray inoculation providing similar amounts of inoculum to plants independent of plant height and used mist irrigation providing uniform humidity to all genotypes. These manipulations may have affected the disease level and weakened the relationship between plant height and FHB. Under natural inoculum pressure and environmental conditions, an stronger negative correlation could have been detected between plant height and FHB. In this study, plant height was not correlated with disease severity in the greenhouse, which supports the results of others (Somers et al. 2003; Klahr et al. 2007). In contrast to field conditions, single-floret inoculation of wheat spikes used in the greenhouse guarantees 100 % inoculation of wheat spikes. In addition, under greenhouse conditions, the inoculated plants, regardless of their height, are subject to infection and disease development as a result of uniform environmental conditions. In the present study, the correlation between plant height and FDK was positive, even though not significant, under field conditions. This observation is different from the results of Couture (1982) who found a significant negative correlation between cultivar height and grain infection by *Fusarium* spp. in wheat.

In addition to passive resistance mechanisms, genetic factors may also determine the negative or positive associations between agronomic traits and FHB. The presence of an overlapping QTL or the pleiotropic effect of a QTL for both agronomic traits and FHB resistance may play an important role in such associations. In a winter wheat population developed from the cross

'Renan' x 'Recital', the overlap of QTLs for resistance to FHB severity on chromosomes 2B and 5A with QTLs for plant height and/or flowering time supported the negative correlation observed between FHB severity and plant height or flowering date (Gervais et al. 2003). In another wheat population derived from 'Wuhan-1'/'Maringa' which later was corrected to 'Wuhan-1'/'Nyu Bai' (McCartney et al. 2007), Somers et al. (2003) detected a QTL on chromosome 2DS for low DON content which coincided with a major gene for plant height. Furthermore, in a population of wheat from a cross between 'Arina' and 'Forno', Paillard et al. (2004) detected FHB resistance QTLs coinciding with heading date and/or plant height QTLs.

There is evidence showing that the presence of the semi-dwarf allele *Rht-D1b* significantly impairs FHB resistance. This allele contributes to significantly increased susceptibility, not due to lower plant height *per se*, but rather to linkage to FHB susceptibility genes in some intervals and/or a pleiotropic physiological effect of the dwarfing allele *Rht-D1b* (Draeger et al. 2007). The association of *Rht-D1b* allele with increased susceptibility to FHB was verified in an independent mapping study based on the population derived from 'Rialto' x 'Spark' which was evaluated under spray-inoculated field conditions (Srinivasachary et al. 2008). In another study, the significant negative effect of *Rht-D1b* on FHB was verified in three non-related segregating winter wheat populations (Voss et al. 2008). In addition, Miedaner and Voss (2008) observed that *Rht-B1d*, *Rht-D1b*, *Rht-B1c*, and *Rht-B1b*+ *Rht-D1b* dwarfing genes significantly increased susceptibility to FHB in wheat. Hilton et al. (1999) revealed that the effects of *Rht-B1b* and *Rht-D1b* were strongly dependent on the genetic background and environmental factors. As mentioned before, there is a possibility of presence of reduced height genes or QTLs for short straw in 'AC Brio', which might have been transferred to the population of RILs in the present study.

Similar to plant height, flowering date was negatively correlated with disease incidence, severity, and DON content under field conditions; early-maturing lines tended to be more diseased than late-maturing lines. A negative association between heading date and FHB also has been reported in several studies (Gervais et al. 2003; Paillard et al. 2004; Schmolke et al. 2005; Häberle et al. 2009). It is generally assumed that the early- or late-maturing lines escape infection by not being at anthesis when optimal conditions are present for infection (Somers et al. 2003) or by slowing down disease spread within the spike when weather conditions are not optimal for disease development (Lin et al. 2006). It is also considered a passive resistance mechanism. Under condition of our experiments, cool weather condition at the end of growing season possibly hampered disease development on late-maturing genotypes. However, a positive correlation was observed between flowering date and FDK under field conditions and between flowering date and disease severity following single-floret inocuations in the greenhouse, Similarly, Steiner et al. (2004) and Somers et al. (2003) found a positive correlation between flowering date and disease spread in the wheat spike, but the correlation was not significant. Several other researchers (Arthur 1891; Steiner et al. 2004; Klahr et al. 2007) also reported a positive correlation between flowering date and FHB under field conditions. The contradictory results for the correlations between flowering date and disease traits may be due to differences in genetic background of the genotypes employed, inoculation techniques, environmental variation, or methods used for disease evaluation. It is possible that flowering date and disease resistance are controlled by different genes/QTLs that are linked, or by pleiotropic effect of a gene/QTL for both groups of traits.

Our results showed that the genotypes with awns, like the 'TC 67' parent, had less FHB than lines without awns under both field and greenhouse conditions. Chrpová et al. (2011) reported that the presence of awns reduced both DON content and FHB index in wheat. Similarly, Snijders (1990) reported a linkage between presence of awns and FHB resistance in winter wheat infected with *Fusarium culmorum* (W. G. Smith) Sacc. However, these findings are different from the results of Mesterházy (1995). He observed that the genotypes with awns were more susceptible to head blight when tested under natural epidemic condition in the field; but this trait did not influence head blight severity in artificial inoculations. Our results also showed a negative association between glume threshability and FHB, indicating that the harder the threshing the lower the FHB development. This finding supports the results of Steiner et al. (2004), but contradicts those of Mesterházy (1995).

The association of FHB resistance with presence/absence of awns and seed threshability also may have a genetic basis. Ban and Suenaga (2000) demonstrated that one of the resistance genes in the FHB-resistant Chinese wheat cultivar 'Sumai 3' may be linked in repulsion to the dominant suppressor *B1* gene for presence/absence of awns. However, Gervais et al. (2003) showed that the FHB resistance QTL located on chromosome 5AL was linked to the *B1* gene in the 'Renan'/'Recital' population. In a winter wheat population developed from the cross 'Dream' x 'Lynx', Schmolke et al. (2005) detected four QTLs for resistance to disease severity, one of which was associated with plant height and spike compactness and another one with flowering date. It should be taken into consideration that separating pleiotropic effects of genes involved in agronomic traits on FHB from the effects of true FHB resistance genes which may be linked to such agronomic traits genes, is not always easy (Buerstmayr et al. 2009).

In this study, the line 'TC 67' derived from *T. timopheevii* demonstrated consistently better resistance to FHB than the susceptible cultivar 'AC Brio'. However, the negative correlation between undesirable agronomic traits and FHB resistance may impede breeding wheat for resistance. Earlier studies showed that it is difficult to combine FHB resistance with excellent agronomic performance (Jiang et al. 2006). However, the low correlations between undesirable agronomic traits and FHB resistance observed in this study indicates that there are opportunities to break any undesirable linkages through selection for the appropriate combination of desired traits and resistance to FHB. In addition, the positive correlation between flowering date and FDK (field) or disease severity (greenhouse) in this study indicates that the selection of genotypes with a combination of desirable flowering date and higher levels of resistance to FHB is possible. Therefore, the line 'TC 67' may be useful to wheat breeders as a new source of FHB resistance if these linkages can be broken.

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