Association of *Fhb1* and *Qfhs.ifa-5A* in Spring versus Winter Growth Habits in Bread Wheat (*Triticum aestivum* L.)

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Abstract

Fusarium head blight (FHB) is a major disease that reduces grain yield and quality of winter and spring wheat in eastern South Dakota. This study was conducted to determine the association of FHB resistance QTLs (*Fhb1* and *Qfhs.ifa-5A*) to spring and winter growth habits of bread wheat. Four genotypes consisting of susceptible winter wheats 'Nekota' and '2137' and moderately resistant spring wheats 'ND2710' and 'BacUp' were crossed and populations derived from the crosses were separated into spring and winter types following cold treatment of seedlings at -7^0C for 1 h. A total of six SSR markers (*Fhb1* markers: Xgwm389, Xgwm493 and STS256; *Qfhs.ifa-5A* markers: Xgwm293, Xgwm304 and Barc186) were used to genotype the populations. A chi-square analysis deviating from a 1:1 ratio showed that there were significant differences in the percentage of genotypes containing homozygous marker alleles for *Fhb1* and *Qfhs.ifa-5A* between spring and winter types in the population ND2710/2137, ND2710/Nekota and BacUp/2137. The percentage of genotypes with homozygous marker alleles for *Fhb1* was lower in the spring types in the populations ND2710/2137 and ND2710/BacUp. In contrast, the spring types in the population ND2710/Nekota had a higher percentage of genotypes containing homozygous marker alleles for *Qfhs.ifa-5A* compared with the winter types. The results indicated that *Fhb1* was not necessarily associated with the spring growth habit; whereas, *Qfhs.ifa-5A* was not necessarily associated with the winter growth habit.

Keywords: Fusarium Head Blight, Quantitative Trait Loci, Marker Assisted Selection, SSR marker, *Fhb1*, *Qfhs.ifa-5A*

1. Introduction

Fusarium head blight (FHB) is a major disease of wheat (*Triticum aestivum*) that reduces yield and end-use quality and produces mycotoxins in the grain (Stack and McMullen 1985; Parry et al. 1995). Because cultural, chemical and biological practices are only partially successful for FHB management, an emphasis has been placed on host-plant resistance. Different sources of FHB resistance have been identified from China, Japan, Eastern Europe, Italy and Brazil. Of these, the Chinese landrace 'Sumai3' and its derivatives are widely used in wheat breeding programs around the world (Rudd et al. 2001). Resistance from Sumai3 and its derivatives is stable and numerous QTLs linked to the FHB resistance have been identified in these sources. A gene, *Fhb1* (formerly *Qfhs.ndsu-3BS*), for FHB resistance was mapped, in Sumai3-derived populations, at 3BS region that explained up to 60% phenotypic variation and reduced disease by about 27% (Liu et al. 2006; Anderson et al. 2007).

Marker-assisted selection (MAS) has been used as a complementary tool in wheat breeding programs focusing on FHB resistance. Del Blanco et al. (2003) verified the efficiency of MAS using *Fhb1* for FHB resistance in a Sumai3-derived population. These authors were able to identify 15 and 10 resistant lines with the aid of SSR markers Xgwm533-3B and Xgwm274-3B, respectively. Zhou et al. (2003) also validated MAS for *Fhb1* in a population derived from Ning7840, a Sumai3 derivative. They found lines with a high level of resistance to the spread of disease when selection was based on phenotype and marker evaluation. Since FHB resistance evaluations are conducted at the adult plant stage, environmental factors affect disease expression in the field. Use of MAS helps to increase gain from selection when combined with evaluating plants at the adult plant stage. Marker assisted selection confirms the presence of genes for resistance, irrespective of expression of phenotype.

Studies have shown the role the physiology or morphology of the plant plays in modification in controlling the level of FHB resistance. Schroeder and Christensen (1963) observed that the anthesis period and percentage of retained and extruded anthers affected FHB resistance levels. Mesterhazy (1995) and Buerstmayr et al. (2000) observed higher disease incidence in awned compared to awnless spikes. Buerstmayr et al. (2000) also reported that plant height was negatively correlated with FHB resistance. Rudd et al. (2001) suggested that genotypes with high humidity around the head would have a high disease. Bread wheat is categorized into spring and winter growth habits based on a vernalization requirement, a period of low temperature inducing flowering. Spring wheat has none or a low vernalization requirement. Winter wheat is planted in the fall and requires exposure to a period of cold temperature prior to initiation of flowering. Four genes, namely *VrnA1*, *VrnB1*, *VrnD1* and *VrnB3* at chromosomes 5AL, 5B, 5DL and 7BS, respectively, were reported to control this vernalization requirement in wheat (Dubcovsky et al. 1998). Gervais et al. (2003) and Paillard et al. (2004) observed that the 5AL QTL for FHB resistance in European winter wheat genotypes was associated with plant height. Buerstmayer et al. (2002) and Somers et al. (2003) detected *Qfhs.ifa-5A* for FHB resistance at 5AS region in CM-82036, a Sumai3-derivative, and Nyu Bai. The possible association of growth habit with *Qfhs.ifa-5A* is not well understood. The objectives of this study were to: i) determine the association of *Fhb1* and *Qfhs.ifa-5A* to spring and winter growth habits, and ii) validate *Fhb1* and *Qfhs.ifa-5A* using marker-assisted selection.

2. Materials and Methods

2.1 Spring and winter growth habit populations

A set of 600 F_2 seeds was grown for selecting plants with either spring or winter growth habits each from the crosses 'Nekota'/'ND2710', '2137'/ND2710 and Nekota/'BacUp'. Nekota (Haley et al. 1996) and 2137 (Sears et al. 1997) are FHB-susceptible winter wheat cultivars, while ND2710 (Frohberg et al. 2004) and BacUp (Busch et al. 1998) are two FHB-resistant spring wheat sources. To select for the spring growth habit, plants were grown without vernalization in a greenhouse maintained at $25 \pm 3^0 C$ and seeds were harvested only from plants which matured 90 d or earlier from planting date. To select for the winter growth habit, the F_2 plants were subjected to cold temperature at the five-leaf stage. Seedlings at the three-leaf stage were vernalized at $4^0 C$ for 8 wk. At the end of the vernalization period, temperature of the soil around the crown root area was decreased to $-7^0 C$ for one h followed by $4^0 C$ for 1 d. Seedlings were transferred into a greenhouse maintained at $25 \pm 3^0 C$ and the tips of the seedlings were cut. Spring types were presumed to be killed by the treatment and seeds were collected from surviving plants. The same two treatments were repeated during the F_3 and F_4 generations and selection continued for spring and winter types. The F_2 derived lines were advanced to the F_5 using the single-seed-descend method.

2.2 Disease evaluation

The $F_{4:5}$ lines were vernalized at 4^0C for 8 wk and transplanted into the field at Brookings, SD (Latitude = $44^016'14''$, Longitude = $-96^046'18''$) in May 2008 in a randomized complete block experimental design with two replications.

An aggressive *Fusarium graminearum* isolate (*Fg4*) was multiplied and macrospores were harvested as described by Zhang et al. (2001). The *F*. isolate was evenly spread on lactic acid (1.6 mL L^{-1}) half-strength potato dextrose agar media. The plates were incubated at 25^0C in a 12 h dark and light cycle for 7 d. Macroconidia were harvested in sterile water. The conidial suspension was adjusted to 70,000 spore mL^{-1} with distilled water. TWEEN 80 (Fisher Scientific International Inc., Pittsburgh, PA) wetting agent was added to the suspension at the rate of 400 μL L^{-1}.

Ten *Fusarium* isolates were used to produce spawn. Maize (*Zea mays* L.) kernels that filled one-fourth of a steel tray (49.5 X 29.2 X 6.4 cm^3) were soaked in water overnight for 12 h. Water was drained and kernels were autoclaved for 45 min. For each isolate, two Petri plates of the previously colonized PDA were cut into approximately 1 cm^2 pieces and spread on each steel tray containing the maize. The inoculated maize was incubated at room temperature for 14 d. The spawn was sun-dried inside a glasshouse for 5 d and stored at 4^0C.

Maize spawn was spread in the field at the rate of 5 g $plot^{-1}$ (1 m by 0.4 m) when wheat was at the jointing stage (Growth Stage (GS) 31, Zadoks et al. 1974) and then at a 7 d interval until heading stage (GS 59). The field nursery was mist-irrigated (0.4 L h^{-1}) on the same day the first spawn was spread in the field. The mist irrigation system was run at the frequency of 2 min every 30 min from 7:00 pm to 7:00 am the following morning. Mist was applied each day until disease ratings were noted for the latest maturing genotype.

At heading, a conidial suspension of 70,000 spore mL^{-1} was applied to spikes using a backpack sprayer (0.5 L min^{-1} at 207 kPa). The suspension was again sprayed 7 d later to inoculate late spikes.

Disease incidence (type I resistance) and severity (type II resistance) were recorded at 21 d after the first conidial suspension application in the field. Late-flowering spikes usually exhibit low disease. Thus, late-flowering plants were not rated by marking the first set of flowering plants with tape. Disease ratings for each entry were averaged over the plants in the plot. Disease incidence was the percentage of number of spikes infected compared with total spikes. Disease severity was the percentage of number of bleached and symptomatic spikelet(s) on each spike. Disease index was calculated as the product of disease incidence and disease severity.

2.3 Genotyping

DNA was extracted from leaf samples following Saghai-Maroof et al. (1984) with minor modifications. Three SSR primers Xgwm389, Xgwm493 (Roder et al. 1998) and STS3B-256 (kindly provided by Dr. J.A. Anderson, University of Minnesota) linked to *Fhb1* were used. The reaction mixture (15 μL) contained 0.2 μM of each primer, 0.2 μM of deoxynucleotide, 2.5 mM $MgCl_2$, 1.5 unit *Taq* polymerase (US DNA Inc, TX) and 200 ng of template DNA. After heating the mixture to 95^0C for 3 min, 40 cycles consisted of 94^0C for 30 sec, 60^0C for 1 min, 72^0C for 1 min and final extension of 72^0c for 10 min. DNA bands were separated by running PCR products on a 4.5% superfine resolution agarose (SFR) gel (Amresco Inc., OH) about 110 v for 4 h.

Three SSR primers Xgwm293, Xgwm304 (Roder et al. 1998) and Xbarc186 (Song et al. 2005) linked to *Qfhs.ifa-5A* were used. A 15 μL PCR mixture consisted of 0.2 μM of reverse primer, 0.2 μM of 6-FAM/VIC/NED/PET-labelled forward primer, 0.2 μM of deoxynucleotide, 1.5 mM $MgCl_2$, 0.25 unit *Taq* polymerase (Invitrogen Corp., CA) and 200 ng of template DNA. After heating the mixture to 95^0C for 3 min, 40 cycles consisted of 94^0C for 30 sec, 60^0C for 1 min, 72^0C for 1 min and a final extension of 72^0C for 10 min. PCR products were scanned with GeneScan-500 LIZ as an internal size standard (Applied Biosystems) in an Applied Biosystems 3130xl (ABI3130xl) DNA Analyzer (Applied Biosystems) and the results were analyzed with GeneMarker software (Softgenetics, LLC.).

2.4 Statistical Analysis

Proc FREQ of SAS (SAS Institute 2008) was used to analyze the data for chi-square test, Chi-square was tested for the equal proportions of resistant to susceptible alleles in spring and winter types.

3. Results

Polymorphic bands were observed for SSR markers Xgwm389, Xgwm493, Xgwm293 and Xgwm304 in the four parents. Polymorphic bands were also detected for the STS3B-256 marker between each of 2137 and Nekota with ND2710 but not with BacUp (Figs. 1 and 2). Similarly, polymorphisms were not detected between Nekota and ND2710 for Xbarc186.

Chi-square analysis showed significant differences in the percentage of spring and winter types carrying *Fhb1* in the Nekota/ND2710 and 2137/ND2710 populations (Table 2). All three SSR markers for *Fhb1* in the 2137/ND2710 population were consistently and significantly lower frequency in the spring types. In contrast, SSR markers Xgwm389 and Xgwm493 showed that lines homozygous for susceptible *Fhb1* were significantly lower in the winter types in the Nekota/ND2710 population. Chi-square analysis did not detect significant differences in the association of *Fhb1* with the 2137/BacUp population.

Significant differences were observed for the association of *Qfhs.ifa-5A* with the winter types (Table 3). A chi-square analysis showed that the percentage of winter types carrying markers Xgwm293 and Xgwm304 for *Qfhs.ifa-5A* was significantly lower than that of the spring types in the Nekota/ND2710 population. Similarly, the association of the resistant *Qfhs.ifa-5A* was lower for winter types in the 2137/BacUp populations, as indicated by patterns of Xgwm304 bands. Chi-square analysis did not detect significant difference in the association of *Qfhs.ifa-5A* with winter type in the 2137/ND2710 population.

Field evaluation showed that lines differed for disease index within the populations. Field transplanting resulted in lower survival rates approaching 11.5% in some lines (Table 4). The distribution of lines homozygous to all markers for resistant or susceptible *Fhb1* or *Qfhs.ifa-5A* alleles are shown in Figs. 3, 4, 5, 6, 7 and 8. The distribution was continuous for resistant alleles. The percentage of lines with resistant alleles for *Qfhs.ifa-5A* exhibiting susceptibility (disease index > 60) ranged from 33.3% to 46.2%, compared with 16.7% to 25% for lines with resistant alleles for *Fhb1*. The distribution of lines with susceptible alleles for *Fhb1* or *Qfhs.ifa-5A* was skewed towards the higher disease index. Average disease index for genotype with homozygous susceptible alleles for *Fhb1* or *Qfhs.ifa-5A* was consistently high (Table 4). Lines with resistant alleles for *Fhb1* and *Qfhs.ifa-5A* had 31.6% to 45.2% and 12.7% to 48.5% lower disease indices, respectively, than lines with susceptible alleles for *Fhb1* and *Qfhs.ifa-5A*. The disease index averaged over the lines containing resistant alleles for both *Fhb1* and *Qfhs.ifa-5A* was low. Simultaneous genotyping with the markers for both *Fhb1* and *Qfhs.ifa-5A* led to identifying resistant (15.1%) and moderately resistant (28.0%) lines in the 2137/ND2710 winter types and Nekota/ND2710 spring types, respectively.

4. Discussion

STS3B-256 is a dominant marker located 0.2 cM from *Fhb1* (Liu et al. 2005). Using a super fine resolution agarose gel enabled the detection of polymorphic bands between each of 2137 and Nekota with ND2710 for the STS3B-256 marker. Detection of polymorphic bands in the agarose gel facilitated genotyping. However, polymorphisms could not be detected between 2137 and BacUp or in their progeny. BacUp and ND2710 were reported to have similar DNA banding patterns for STS3B-256 (personal communication with Sixin Liu). Additional genotyping using the Nekota/BacUp population showed that BacUp was different from ND2710. This result suggests that the STS3B-256 markers produced different DNA band sizes for BacUp and ND2710.

Though an original 600 individual F_2 population was utilized to develop spring and winter types in each of the four spring-by-winter wheat crosses, three cycles of cold treatments resulted in the recovery of only few winter types from these crosses. Not a single line with a winter growth habit was recovered in the Nekota/BacUp population; hence the whole population was discarded from the final analysis.

Significant variability was observed for the association of *Fhb1* and *Qfhs.ifa-5A* in the spring and winter wheat types. The percentage of resistant spring types in the 2137/ND2710 population was very low for all *Fhb1* SSR markers. Similarly, the percentage of susceptible winter types was low in the Nekota/ND2710 population for SSR markers Xgwm389 and Xgwm493. Overall the results indicated that the percentage of resistant lines was higher within the winter type and lower within the spring type, as measured by markers associated with *Fhb1*. On the other hand, association of *Qfhs.ifa-5A* was higher for resistant lines within the spring type. The association of *Fhb1* and *Qfhs.ifa-5A* differed for spring and winter wheat types, and this difference was found to be population-specific.

This study showed marker assisted selection was effective when selecting homozygous genotypes for both *Fhb1* and *Qfhs.ifa-5A* markers. Individually, selecting genotypes homozygous for all *Fhb1* markers was effective as the markers were able to identify susceptible genotypes. Selection based on *Fhb1* markers would reduce the average disease index of the population by at least 31.6%. This agrees with Pumphrey et al. (2007) who reported a 27% reduction in disease expression when genotypes were selected for *Fhb1*. Compared to *Fhb1*, selecting genotypes based on *Qfhs.ifa-5A* was less efficient. The markers for *Qfhs.ifa-5A* were less effective in detecting homozygous resistant from susceptible genotypes in the different populations. Buerstmayr et al. (2002, 2003) reported that *Qfhs.ifa-5A* was linked to disease incidence (type I resistance) rather than severity (type II resistance). Both ND2710 and BacUp are presumed to have type II resistance. Since type II resistant parents

were used in this study, the effect of *Qfhs.ifa-5A* might not have been as distinct as *Fhb1* in the populations. In the different populations, markers for *Fhb1* and *Qfhs.ifa-5A* when combined were effective in detecting resistant or moderately resistant genotypes; thus validating the effectiveness of marker-assisted selection for FHB resistance.

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Table 1. Pedigree and seed source of the parental genotypes used in this study

Parents	Pedigree	
ND2710	'Sumai3'/'Wheaton'/ /'Grandin'	Frohberg et al., 2004
BacUp	'Nuy Bay'/'2375//'Marshall'	Busch et al., 1998
2137	W2440/W9488//'2163'	Sears et al., 1997
Nekota	'Bennett'/'TAM 107'	Haley et al., 1996

Table 2. Chi-square value (χ^2) and percent resistant and susceptible populations for *Fhb1* within spring and winter growth habits in the crosses 2137/ND2710, Nekota/ND2710, 2137/BacUp

Population	Growth Habit	Xgwm389			Xgwm489			STS3B-256		
		A	B	χ^2	A	B	χ^2	A	B	χ^2
2137/ND2710	Spring	73.0	25.2	15.0**	70.1	22.2	18.1**	77.1	22.9	20.9**
	Winter	43.6	49.1		38.6	47.4		45.6	54.4	
Nekota/ND2710	Spring	44.4	48.6	13.9**	56.3	43.7	4.8*	59.7	40.3	1.1[NS]
	Winter	22.2	77.8		33.3	50.0		66.7	33.3	
2137/BacUp	Spring	56.4	34.0	2.4[NS]	69.5	27.4	2.1[NS]	_	_	_
	Winter	65.5	24.1		62.1	37.9		_	_	

A = homozygous susceptible genotypes either 2137 or Nekota; B = homozygous resistant genotypes either ND2710 or BacUp

[NS] = non significant

*,** = significant at 0.05 and 0.01 probability level, respectively

Table 3. Chi-square value (χ^2) and percent resistant and susceptible populations for *Qfhs.ifa-5A* spring and winter growth habits in the crosses 2137/ND2710, Nekota/ND2710, 2137/BacUp

Population	Growth Habit	Xgwm293			Xgwm304			Barc186		
		A	B	χ^2	A	B	χ^2	A	B	χ^2
2137/ND2710	Spring	45.3	44.4	0.5[NS]	44.9	44.9	0.4[NS]	41.0	43.6	0.5[NS]
	Winter	51.8	41.1		49.1	40.4		46.4	39.3	
Nekota/ND2710	Spring	36.2	56.5	14.8[**]	37.1	55.7	13.8[**]	–	–	–
	Winter	66.7	33.3		66.7	33.3		–	–	–
2137/BacUp	Spring	49.5	34.7	3.7[NS]	46.9	38.5	5.5[*]	46.9	35.4	2.3[NS]
	Winter	70.0	26.7		64.3	25.0		65.5	31.0	

A = homozygous susceptible genotypes (2137 or Nekota); B = homozygous resistant genotypes (ND2710 or BacUp)

[NS] = non significant

[*], [**] = significant at 0.05 and 0.01 probability level, respectively.

Table 4. FHB disease index for resistant and susceptible genotypes with Xgwm389, Xgwm493, STS3B-256, Xgwm293, Xgwm304 and Xbarc186 markers in the spring and winter growth habits from 2137/ND2710, Nekota/ND2710 and 2137/BacUp in 2008 in the field environment

Population	Growth Habit	Allele	No. of Lines†	Disease Index (DI)										
				Xgwm-389	Xgwm-493	STS3B-256	*Fhb1*‡	% Reduction§	Xgwm-293	Xgwm-304	Xbarc-186	*Qfhs.ifa-5A*‡	% Reduction§	*Fhb1+ Qfhs.ifa-5A*‡
2137/ND2710	Spring	2137	28	68.4	66.1	67.0	68.0		65.3	67.5	71.7	67.0		79.8
		ND2710		52.6	52.3	49.0	46.5	31.6	58.5	58.5	58.5	58.5	12.7	47.7
	Winter	2137	26	71.9	76.0	82.6	85.6		74.1	75.7	77.6	77.6		100.0
		ND2710		66.1	57.6	60.6	46.9	45.2	56.5	56.5	57.7	57.7	25.6	15.0
Nekota/ND2710	Spring	Nekota	43	73.5	70.3	68.8	71.6		73.1	73.1	-	73.1		73.1
		ND2710		53.4	58.5	61.8	48.0	33.0	58.1	58.1	-	58.1	20.5	44.1
	Winter	Nekota	2	-	-	-	-	-	-	-	-	-	-	-
		ND2710		-	-	-	-	-	-	-	-	-	-	-
2137/BacUp	Spring	2137	11	95.8	90.9	-	95.8		83.0	80.9	80.9	80.9		95.4
		BacUp		69.5	55.9	-	55.9	41.6	-	-	-	-	-	-
	Winter	2137	12	68.4	76.0	-	73.0		70.1	70.2	67.8	70.2		78.4
		BacUp		41.7	45.7	-	41.7	42.9	36.3	36.3	55.4	36.3	48.3	15.1

† survived after transplanting

‡ homozygous for all markers

§ % Reduction = |(Homozygous resistant DI – Homozygous susceptible DI)| * 100 / Homozygous susceptible DI
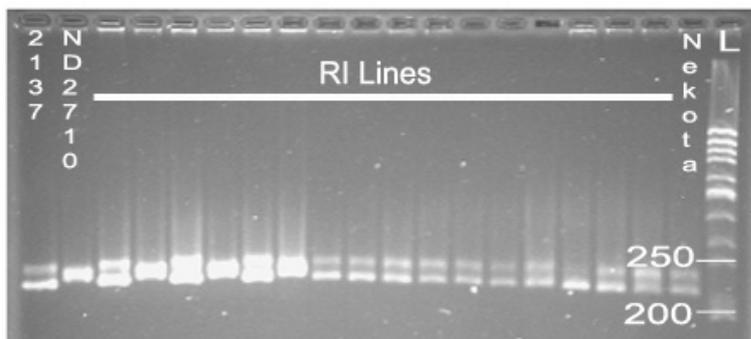
Figure 1. Separation of polymerase chain reaction products amplified by STS3B-256 on a super fine resolution agarose gel (3.5%, 110 V, 4 h). DNA samples bands were '2137', 'ND2710', recombinant inbred lines (RI Lines) from cross '2137'/'ND2710', 'Nekota' and DNA ladder (L) (50bp, Fisher Scientific International Inc.)
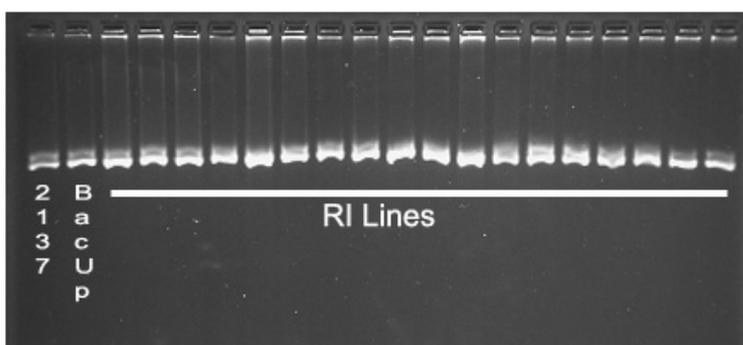
Figure 2. Separation of polymerase chain reaction products amplified by STS3B-256 on a super fine resolution agarose gel (4.0%, 95 V, 4 h). DNA samples bands were '2137', 'BacUp' and recombinant inbred lines (RI Lines) from cross '2137'/'BacUp'
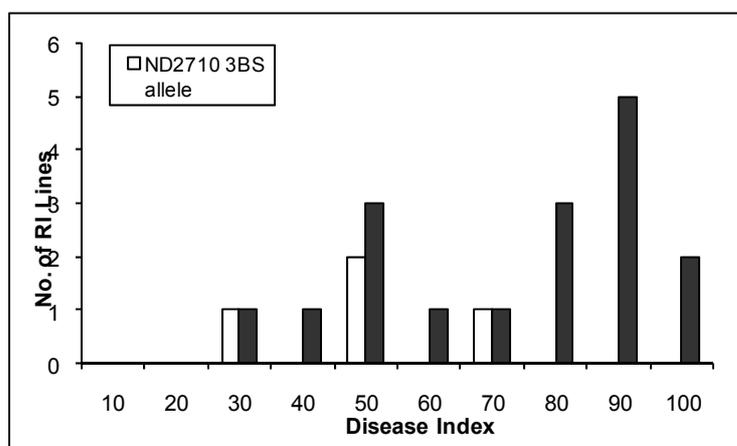
Figure 3. Frequency distribution of FHB disease index for spring growth types recombinant inbred (RI) lines with ND2710 or 2137 alleles homogyzous for three marker Xgwm389, Xgwm493 and STS3B-256
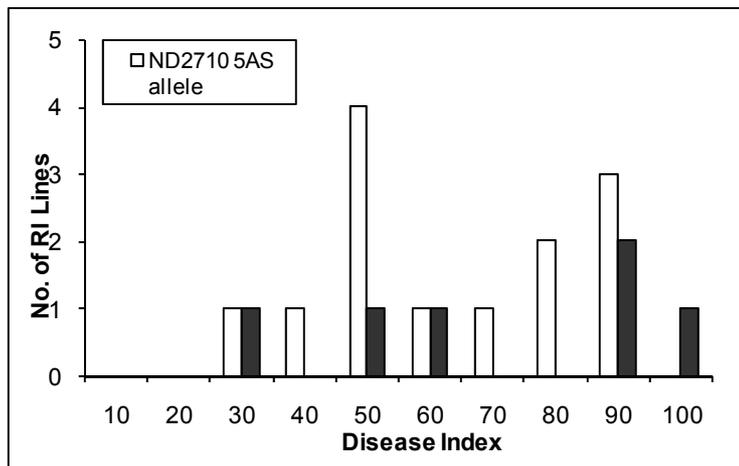
Figure 4. Frequency distribution of FHB disease index for spring growth types recombinant inbred (RI) lines with ND2710 or 2137 alleles homogyzous for three marker Xgwm293, Xgwm304 and Xbarc186
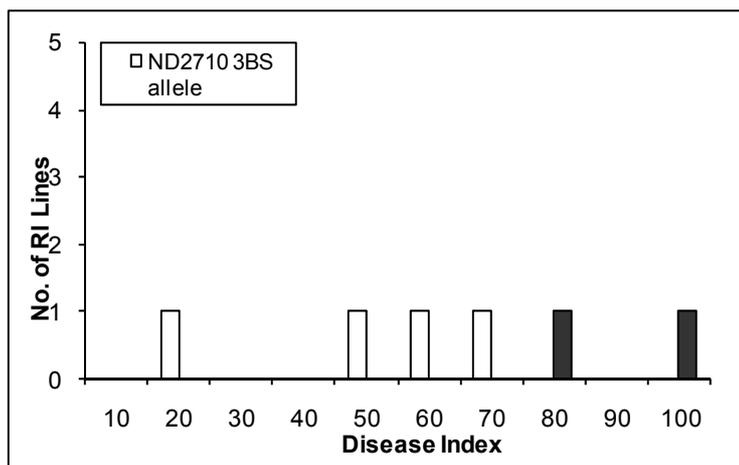
Figure 5. Frequency distribution of FHB disease index for winter growth types recombinant inbred (RI) lines with ND2710 or 2137 alleles homogyzous for three marker Xgwm389, Xgwm493 and STS3B-256
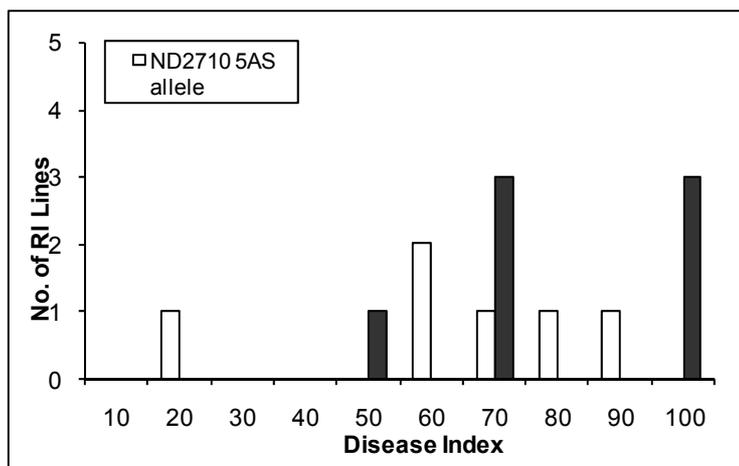
Figure 6. Frequency distribution of FHB disease index for winter growth types recombinant inbred (RI) lines with ND2710 or 2137 alleles homogyzous for three marker Xgwm293, Xgwm304 and Xbarc186
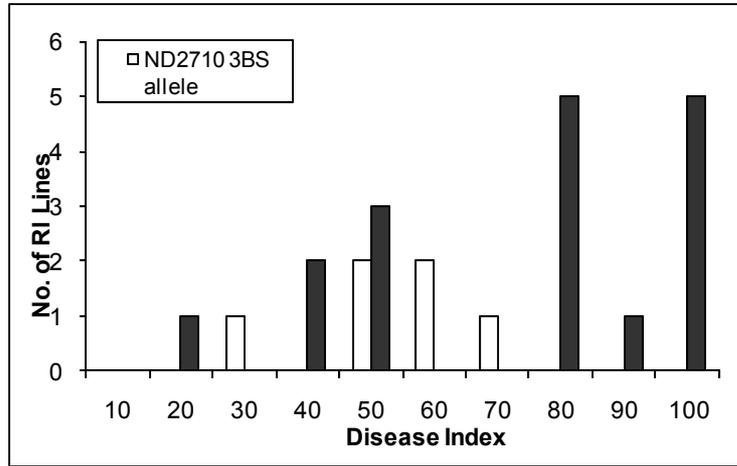
Figure 7. Frequency distribution of FHB disease index for spring growth types recombinant inbred (RI) lines with ND2710 or Nekota alleles homogyzous for three marker Xgwm389, Xgwm493 and STS3B-256
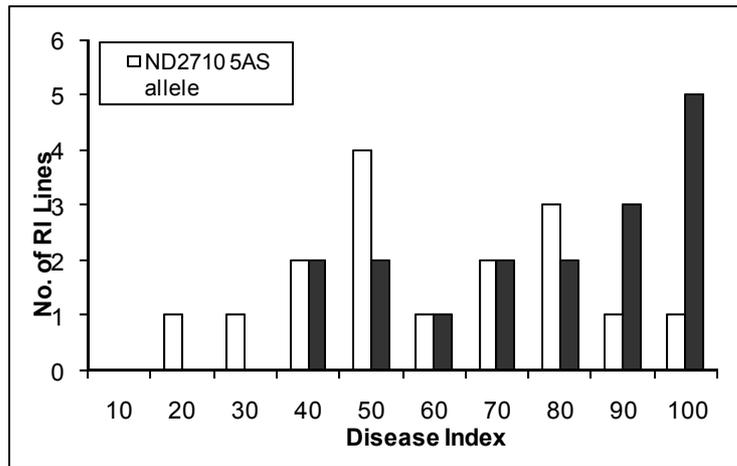
Figure 8. Frequency distribution of FHB disease index for spring growth types recombinant inbred (RI) lines with ND2710 or Nekota alleles homogyzous for three marker Xgwm29 and Xgwm304