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# Registration of Near-Isogenic Winter Wheat Germplasm Contrasting in *Fhb1* for Fusarium Head Blight Resistance

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## ABSTRACT

**The Chinese wheat germplasm Ning7840 (*Triticum aestivum* L.) contains *Fhb1*, a quantitative trait locus (QTL) for Fusarium head blight (FHB) resistance that explains as much as 53% of the phenotypic variation in segregating populations. Ning7840 has been widely used as a resistant parent in breeding programs worldwide, but because of its poor adaptation in the United States, its progenies usually exhibit reduced grain yield due to the transfer of undesirable genes from Ning7840. The development of five near-isogenic lines (NILs: 'Clark'*7/Ning7840) (NIL75, Reg. No. GS-174, PI 668559; NIL78, Reg. No. GS-175, PI 668560; NIL80, Reg. No. GS-176, PI 668561; NIL90, Reg. No. GS-177, PI 668562; NIL98, Reg. No. GS-178, PI 668563) contrasting in *Fhb*1 alleles was intended to overcome this potential limitation. Marker-assisted backcrossing was used to develop the NILs. Approximately 2000 $BC_7F_2$ plants from the backcross of Ning7840 by Clark (recurrent parent) were screened with two *Fhb1*-flanking markers (*Xgwm533* and *Xgwm493*), and selected $BC_7F_3$ families were evaluated for FHB resistance in greenhouses. Both genotypic and phenotypic data confirm the presence of *Fhb1* in the four resistant NILs and absence in the one susceptible NIL. All resistant NILs had significantly higher FHB resistance and lower deoxynivalenol content than Clark and the susceptible NIL but yield similar to Clark ($P = 0.295$). Marker-assisted backcross efficiently transferred *Fhb1* into U.S. hard winter wheat without transferring undesirable traits from Ning7840, and these *Fhb1* NILs should be useful parents for effective use of *Fhb1* in U.S. winter wheat.**



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Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe; teleomorph *Gibberella zeae* (Schwein.:Fr.) Petch, is a destructive disease that reduces wheat (*Triticum aestivum* L.) grain yield and quality. Breeding varieties with FHB resistance is the most effective approach to controlling FHB. Resistance to spread of disease within a spike (Type II resistance) is considered a stable form of FHB resistance, and *Fhb1* on chromosome 3BS is the major quantitative trait locus (QTL) governing Type II resistance (Buerstmayr et al., 2009). Ning7840, a derivative of 'Sumai3', has a high level of Type II resistance, and its *Fhb1* explains as much as 53% of phenotypic variation in segregating populations (Bai et al., 1999; Liu et al., 2009). In addition, Ning7840 is resistant to leaf (*Puccinia triticina* Eriks.) and stripe (*Puccinia striiformis* f. sp. *tritici*) rusts and powdery mildew (*Blumeria graminis* f. sp. *tritici*) and has better agronomic characteristics than Sumai3 (Bai et al., 1989). For these reasons, Ning7840 has been used frequently as a source of resistance in breeding programs (Lu and Wang, 1991). However, the direct use of unadapted Ning7840 as a resistant parent in U.S. breeding programs often results in grain yield reduction. Breaking the association between *Fhb1* and other unadapted traits is critical for successful use of *Fhb1* in U.S. winter wheat breeding programs. Here, we report the development and characterization of near-isogenic lines (NILs) carrying *Fhb1* in U.S.-adapted winter wheat cultivar Clark.

## Materials and Methods

Ning7840 ('Aurora'/'Anhui11'//Sumai3) is a hard red facultative Chinese elite breeding line that carries *Fhb1* and shows high resistance to FHB. Clark [PI 512337, 'Beau'//(65256A1-8-1/67137B5-16/4/'Sullivan'/3/Beau//5517B8-5-3-3/'Logan')] is a soft winter wheat variety released from Purdue University, IN; it has high yield potential but is susceptible to FHB (Ohm et al., 1988). $F_1$ plants from the cross Ning7840/Clark were backcrossed to Clark seven times. Approximately 2000 $BC_7F_2$ plants were screened with flanking markers *Xgwm533* (forward

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**Abbreviations:** FHB, Fusarium head blight; NIL, near-isogenic line; PCR, polymerase chain reaction; PSS, proportion of symptomatic spikelets; QTL, quantitative trait locus.

primer 5′-AAGGCGAATCAAACGGAATA-3′; reverse 5′-ATAAGGCAAACTAAGCGGAA-3′) and *Xgwm493* (forward primer 5′-TTCCCATAACTAAAACCGCG-3′; reverse 5′-GCGCCAAAATCAATACCCTT-3′) for *Fhb1*, and selected $BC_7F_3$ families were evaluated for FHB resistance in greenhouses. Ten highly resistant lines carrying homozygous *Fhb1* and five highly susceptible lines without *Fhb1* were further evaluated for FHB resistance and advanced to the $BC_7F_7$ generation. Five NILs (NIL75, Reg. No. GP-174, PI 668559; NIL78, Reg. No. GP-175, PI 668560; NIL80, Reg. No. GP-176, PI 668561; NIL90, Reg. No. GP-177, PI 668562; NIL98, Reg. No. GP-178, PI 668563)—four most-resistant NILs and one most-susceptible NIL—among the 15 NILs (Clark*7/Ning7840) were finally selected at $BC_7F_7$ based on phenotypic data and validated for the presence and absence of markers for *Fhb1*. In addition, these NILs were genotyped with 168 markers distributed across all 21 chromosomes to assess for residual Ning7840 genetic background. A 10-μL polymerase chain reaction (PCR) mix contained 1× ammonium sulfate buffer (Bioline, Randolph, MA), 2.5 mM $MgCl_2$, 200 μM of each dNTP, 50 nM of forward tailed primer, 100 nM of reverse primer, 50 nM of M13-dye-labeled fluorescent primer, 100 ng DNA, and 1 unit *Taq* polymerase (Promega, Madison, WI). Polymerase chain reaction was performed in a PTC-200 thermal cycler (Bio-Rad Labs, Hercules, CA) using a touchdown program starting at 96°C for 5 min; followed by 5 cycles of 1 min at 96°C, 5 min at 68°C minus 2°C per cycle, and 1 min at 72°C; 5 cycles of 1 min at 96°C, 2 min at 58°C minus 2°C per cycle, and 1 min at 72°C; 25 cycles of 1 min at 96°C, 1 min at 50°C, and 1 min at 72°C; and a final extension of 5 min at 72°C. Primers labeled with FAM, VIC, NED, or PET (Life Technologies, Grand Island, NY) were run as described by Bernardo et al. (2013). Fluorescent dye-labeled PCR products were visualized on an ABI3730 sequencer (Life Technologies) and were scored using GeneMarker V1.5 (SoftGenetics, State College, PA).

The selected NILs and Clark were evaluated for FHB resistance in both field and greenhouse by single-floret inoculation. *Fusarium graminearum* conidia (strain GZ 3639, a Kansas field isolate) were produced according to Bai et al. (2000). Plants were grown in the greenhouse at 25°C for 12 h of light and at 18°C for 12 h of darkness. At anthesis, a 10-μL (~1,000 spores) *F. graminearum* conidiospore suspension was injected into a central spikelet of a spike. Inoculated plants in the greenhouse were enclosed in a plastic chamber with 100% humidity for 48 h to initiate FHB infection. Disease rating was done 21 d after inoculation, and the proportion of symptomatic spikelets (PSS) per spike was recorded as the percentage of infected spikelets in a spike calculated by dividing the number of infected spikelets by the total number of spikelets in a spike.

Yield trials of NILs and Clark were conducted using a randomized plot design with three replications in 2010 (Manhattan, KS) and 2011 (Manhattan, KS, and Urbana, IL). Analysis of variance was conducted on grain yield and disease severity across locations with SAS version 9.2 (SAS Institute, Cary, NC).

# Characteristics

## Genotypes of NILs

The NILs were fingerprinted using 168 SSR markers across all 21 chromosomes to confirm the presence of *Fhb1* and to determine residual genetic background from Ning7840. All four FHB-resistant NILs have the 3BS region harboring *Fhb1* from Ning7840; three of them, NILs 78, 80, and 90, also contain a Ning7840 fragment harboring *Xbarc108* and *Xwmc603* in chromosome 7A. These two markers are close to a previously identified QTL, *Fhb7AC*, in Sumai3 (Jayatilake et al., 2011). In addition, NIL80 contains Ning7840 alleles for markers *Xgwm147* (1DS) and *Xwmc311* (7BL); NIL90 has three Ning7840 fragments in chromosomes 1BL, 1DL, and 2BL; and NIL75 contains four Ning7840 fragments in chromosomes 1DL, 2BL, 7AL, and 7DL. Linked QTLs have not been reported for these markers in Sumai3 or Ning7840 (Liu et al., 2009). The susceptible NIL (NIL98) showed Clark alleles for all markers tested except for *Xwmc603*. Thus, marker-assisted backcross successfully transferred the *Fhb1* into the Clark background.

## FHB Resistance

Four resistant and one susceptible NIL (NIL98) were selected for FHB evaluation in both greenhouse and field experiments based on the presence or absence of resistance alleles of *Fhb1* flanking markers *Xgwm533* and *Xgwm493*. The mean PSS in all resistant NILs was low, ranging from 7 to 30% in the greenhouse and 11 to 16% in the fields, whereas PSS for NIL98 (95% in greenhouse and 53% in the fields) and Clark (89% in the greenhouse and 62% in the fields) were high (Table 1). The difference in PSS between resistant NILs and Clark is significant at $P < 0.05$ (Table 1) but insignificant between the susceptible NIL and Clark. Although marker data indicated the presence of a fragment from 7A close to a previously identified *Fhb7AC* in Sumai3, it is unlikely that this QTL contributed to FHB resistance in these NILs because NIL98 containing the fragment was highly susceptible to FHB, and NIL75 without

**Table 1. Proportion of symptomatic spikelets in a spike (% PSS) for the five wheat near-isogenic lines (NILs) and Clark evaluated under greenhouse (Manhattan, KS) and field conditions (Manhattan, KS, and Urbana, IL).**

| Line | Greenhouse Manhattan | Field 2010 Manhattan | Field 2011 Manhattan | Field 2011 Urbana | Field mean |
|---|---|---|---|---|---|
| NIL75 | 7 | 10 | 5 | 16 | 11a† |
| NIL78 | 15 | 14 | 5 | 18 | 13a |
| NIL80 | 30 | 10 | 5 | 26 | 15a |
| NIL90 | 13 | 16 | 5 | 27 | 16a |
| NIL98 | 95 | 77 | 42 | 66 | 53b |
| Clark | 89 | 76 | 49 | 66 | 62b |

† Means followed by the same letter are not significantly different at $P < 0.05$; Dunnett's critical value = 12.5, CV = 35.22%.

the fragment from Ning7840 showed the highest resistance among the resistant NILs (Table 1).

## Agronomic Evaluation

Results from the 2011 Illinois field trial (Table 2) showed that the NILs headed 1 to 2 d earlier or at the same time as Clark, were within a 1.8 kg $hL^{-1}$ range of Clark's test weight, and had a height variation of 5 cm. The overall agronomic performance of the resistant NILs was comparable to that of Clark, but seeds of resistant NILs had lower deoxynivalenol content than the susceptible NIL and Clark. Grain yields of the NILs were not significantly different ($P = 0.278$) from that of Clark (Table 3); therefore, *Fhb1* per se is not associated with undesirable agronomic traits, and the resistant NILs developed in this study should be a good source of *Fhb1* for integration of *Fhb1* into U.S. winter wheat.

## Availability

Small amounts of seed are available for distribution to wheat breeders, geneticists, and other research personnel on written request to the corresponding author. Seed of the NILs has been deposited in the National Plant Germplasm System, where it will be available for 5 years after the date of publication for research purposes, including development and commercialization of new varieties. Appropriate recognition of the source is requested if these germplasm lines contribute to the development of a new breeding line or variety.


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**Table 2. Agronomic performance of the five wheat near-isogenic lines (NILs) and Clark in 2011 Illinois field trial.**

| Line | Test wt. | Heading date | Height | DON† |
|---|---|---|---|---|
| | kg $hL^{-1}$ | after 30 Apr. | cm | mg $kg^{-1}$ |
| NIL75 | 69.37 | 13 | 104.1 | 3.7 |
| NIL78 | 71.69 | 13 | 99.1 | 3.7 |
| NIL80 | 71.04 | 12 | 101.6 | 3.7 |
| NIL90 | 70.27 | 11 | 101.6 | 3.9 |
| NIL98 | 70.79 | 12 | 96.5 | 9.8 |
| Clark | 71.17 | 13 | 99.1 | 9.8 |

† DON, deoxynivalenol.

**Table 3. Yield of the five wheat near-isogenic lines (NILs) and Clark evaluated in Manhattan, KS (2010, 2011) and Urbana, IL (2011).**

| Line | 2010 KS | 2011 KS | 2011 IL | Mean |
|---|---|---|---|---|
| | kg $ha^{-1}$ | | | |
| NIL75 | 1890.14 | 2707.66 | 2379.36 | 2325.75 |
| NIL78 | 2292.71 | 2737.80 | 2961.69 | 2664.07 |
| NIL80 | 1886.91 | 2527.90 | 2769.02 | 2394.61 |
| NIL90 | 1855.16 | 2517.14 | 2505.84 | 2292.71 |
| NIL98 | 1938.04 | 3201.73 | 2929.40 | 2689.72 |
| Clark | 2011.24 | 2580.65 | 2875.04 | 2488.97 |


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