Chapter 36
Determining the Order of Resistance Genes *Qsng-3BS*, *Fhb1* and *Sr2* and Combining Them in Coupling on Wheat Chromosome 3BS

Rima Thapa, Gina Brown-Guedira, Herbert W. Ohm, Kiersten Wise, and Stephen B. Goodwin

Abstract A variety of diseases of wheat (*Triticum aestivum* L.) occurs every year in the U.S. leading to significant grain yield losses. *Stagonospora nodorum* blotch (SNB), fusarium head blight (FHB) and stem rust (SR) are caused by the fungi *Stagonospora nodorum, Fusarium graminearum* and *Puccinia graminis*, respectively. These diseases penalize both grain yield and quality. Three resistance factors, *Qsng.sfr-3BS*, *Fhb1* and *Sr2* conferring resistance, respectively, to SNB, FHB and SR, each from a unique donor wheat parent line, have been mapped to chromosome 3BS of wheat and are believed to be closely linked. Based on previously published analyses, *Sr2* is on the distal end, *Fhb1* is on the proximal end and *Qsng.sfr-3BS* is in the middle of *Sr2* and *Fhb1* in the 3BS wheat genome. Thus, the objectives of this project are to determine the gene order of *Qsng.sfr-3BS*, *Fhb1* and *Sr2*, in a linkage block on chromosome 3BS and combining them in coupling. The linkage relationships were determined through analysis of a three-way cross between parental lines Arina, Alsen and Ocoroni86, containing the resistance genes *Qsng.sfr-3BS*, *Fhb1* and *Sr2,* respectively. A total of 1,600 F_2 plants was screened, along with the parental lines, using KASPar genotyping technology via single-nucleotide polymorphism markers to identify the recombinant progeny. Phenotypic screening for SNB was performed on the entire F_2 population. Knowing the positional order of these

R. Thapa (✉) • H.W. Ohm
Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA
e-mail: rthapa@purdue.edu

G. Brown-Guedira
USDA-ARS, Crop Science Department, North Carolina State University, Box 7620, Raleigh, NC 27695, USA

K. Wise
Department of Botany and Plant Pathology, Purdue University, 915 West State Street, West Lafayette, IN 47907-2054, USA

S.B. Goodwin
Crop Production and Pest Control Research Unit, USDA-ARS, Purdue University, 915 West State Street, West Lafayette, IN 47907-2054, USA

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resistance genes will enable the development of a wheat line with three genes in coupling to provide durable and broad-spectrum resistance against three major diseases of wheat.

Introduction

Stagonospora nodorum blotch (SNB), fusarium head blight (FHB) and stem rust (SR) of wheat are caused by the fungi *Stagonospora nodorum*, *Fusarium graminearum* and *Puccinia graminis*, respectively. Each of these diseases can cause yield losses up to 50 % or more during severe epidemics and when environmental conditions are favorable (Chester 1943; Roefls 1978; Stakman and Harrar 1957; Wicki et al. 1999). In addition to yield reduction, FHB reduces quality due to production of a mycotoxin called vomitoxin (deoxynivalenol) produced by *F. graminearum* (Bai et al. 2001; Gilbert and Tekauz 2000), which is harmful to both humans and livestock. FHB also reduces test weight and lowers market grade. Thus, FHB is one of the most feared fungal diseases of wheat because an entire crop can be rejected for human consumption due to mycotoxin contamination. SR has a capacity of destroying millions of hectares of healthy, high-yielding wheat in less than a month by reducing fields to a mass of bare stalks supporting only small, shriveled grains by harvest time (Singh et al. 2008). There have been several epidemics of SR during the past 80 years that have reduced the yield by 50 % in the Great Plains (Chester 1943; Roefls 1978; Stakman and Harrar 1957). SNB is one of the major foliar and glume diseases of wheat and the most yield loss occurs when the flag leaf and the two leaves below the flag leaf become infected by the time the wheat flowers in late May.

The objective of this study is to find the gene order of *Fhb1*, *Sr2* and *Qsng.sfr-3BS* with the long-term goal of combining them in a linkage block on wheat chromosome 3BS. The first objective was achieved by crossing three unique parental lines with resistance genes *Fhb1*, *Sr2* and *Qsng.sfr-3BS* to combine them into one background. An F_2 population segregating for all three genes was genotyped with single-nucleotide polymorphism (SNP) markers to validate the presence of markers linked to the resistance genes and also to determine the gene order. The F_2 population was also phenotyped for level of resistance to SNB.

Materials and Methods

The mapping population consisted of 1,600 F_2 progeny derived from a three-way cross between wheat cultivars Arina, Alsen and Ocoroni86 providing the resistance genes *Qsng.sfr-3BS, Fhb1,* and *Sr2*, respectively. The F_2 population, its parents and cultivar Chinese Spring as negative control, were planted in January, 2013 in a greenhouse at Purdue University in West Lafayette, Indiana, U.S.A.. The primary

spike after spike emergence (Feekes growth stage 10.3) of an adult plant of every F_2 progeny and the parental lines was phenotyped in a greenhouse using an isolate of *S. nodorum* obtained from an infected wheat field in Indiana. The disease severity scores were recorded 21 days after inoculation on a 0–9 scale. The F_2 plants were screened along with the parental lines using KASPar genotyping technology to identify the recombinant plants. Single-nucleotide polymorphism markers were utilized to identify the recombinants, determine the gene order, to make the genetic map and for quantitative trait loci (QTL) analysis. Linkage analysis was performed and the map was made with JoinMap 3.0.

Results and Discussion

Phenotyping of all 1,600 F_2 progeny was performed and the results utilized the full range of the 0–9 disease-rating scale. The frequency distribution of the percent diseased glume tissue of F_2 progeny derived from the three-way cross of Arina (containing the *Qsng.sfr-3BS* SNB resistance QTL), Alsen and Ocoroni86 inoculated with a field isolate of *S. nodorum* in a greenhouse showed a continuous distribution with a skew towards susceptibility, indicating the presence of one or more QTL for resistance (data not shown).

The preliminary genetic linkage map suggests that the stem rust resistance gene (*Sr2)* is the most distal, the fusarium head blight resistance gene (*Fhb1)* is proximal, and with the *S. nodorum* resistance gene (*Qsng.sfr-3BS)* is between *Sr2* and *Fhb1* on chromosome 3BS of wheat (Fig. 36.1). This result supports our hypothesis made

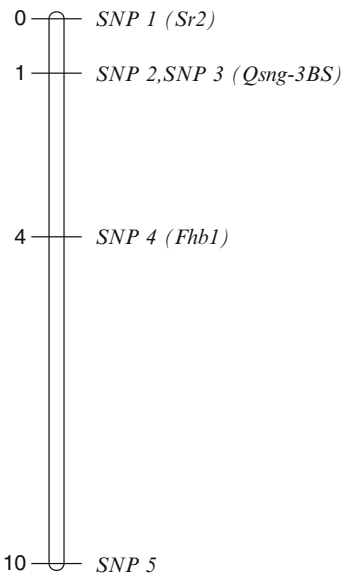

Fig. 36.1 Preliminary linkage map of wheat chromosome 3BS for markers SNP 1, SNP 2, SNP 3, SNP 4, and SNP 5 in the F2 population of 1,600 individuals derived from a three-way cross of wheat cultivars Arina, Alsen and Ocoroni86. The map was generated with JoinMap 3.0 at LOD = 9.0. Numbers to the *left* of the vertical bar indicate the total distance in centimorgans, and positions of mapped markers are indicated on the *right*. The approximate positions of disease resistance genes are indicated by *brackets*

from analyses of previously published marker positions. Knowing the correct order and relative distance between the three resistance genes indicates how a linkage block can be created. Markers for *Sr2* and *Qsng.sfr-3BS* were placed 1 cM apart in our mapping population while the predictive marker for *Fhb1* was located 4 cM proximal from *Sr2*. Thus, recombinant plants having both *Sr2* and *Qsng.sfr-3BS* were recovered at a lower frequency than plants having *Fhb1* and *Qsng.sfr-3BS* in coupling. These plants having two resistance genes in coupling will be used in further crosses to obtain recombinants having all three resistance genes in a linkage block. The tight linkage between *Sr2*, *Qsng.sfr-3BS*, and *Fhb1* indicates that it should be easy to maintain this linkage block in a breeding program.

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