Theor Appl Genet (2003) 106:1027–1031
DOI 10.1007/s00122-002-1137-4

I. A. del Blanco · R. C. Frohberg · R. W. Stack
W. A. Berzonsky · S. F. Kianian

Detection of QTL linked to Fusarium head blight resistance in Sumai 3-derived North Dakota bread wheat lines

Received: 22 May 2002 / Accepted: 23 August 2002 / Published online: 2 November 2002

Abstract During the past decade Fusarium head blight (FHB) caused by *Fusarium graminearum* Schwabe has resulted in severe grain yield and quality losses of wheat (*Triticum aestivum* L.) in the Northern Great Plains of the U.S. Given the complexity of breeding for FHB resistance, molecular markers associated with this trait will be valuable in accelerating efforts to breed resistant cultivars. The objective of this study was to identify molecular markers linked to quantitative trait loci (QTL) for FHB resistance in wheat using a set of lines obtained by several cycles of crossing to North Dakota adapted genotypes, which derived their resistance from cv. Sumai 3. Microsatellite markers spanning the wheat genome were used to screen parents and derived lines. Polymorphisms for parental alleles were compared to disease scores for Type II resistance. The probability of linkage between markers and introgressed resistance genes was calculated using a binomial probability formula based on the assumption that a molecular marker at a specific distance from the introgressed gene, in a near-isogenic line (NIL), will carry the donor-parent allele as a function of the distance between marker and gene and the number of backcrosses/selfs performed in deriving the NIL. Microsatellite loci *Xgwm533* and *Xgwm274* were significantly associated with QTL for FHB resistance.

Keywords Wheat · Scab · *Fusarium graminearum* · QTL · Molecular markers · Association mapping

Communicated by J. Dvorak

I.A. del Blanco · R.C. Frohberg · W.A. Berzonsky
S.F. Kianian (✉)
Plant Sciences Department, Loftsgard Hall,
North Dakota State University, Fargo, ND 58105-5051, USA
e-mail: s.kianian@ndsu.nodak.edu
Fax: +1-701-2318474

R.W. Stack
Plant Pathology Department, Walster Hall,
North Dakota State University, Fargo, ND 58105-5051, USA

Introduction

Genetic studies to identify markers linked to quantitative trait loci (QTL) require the development of population structures such as recombinant inbred lines (RIL), near-isogenic lines (NIL), double haploid lines, or backcross-derived lines, and the genotyping and phenotyping of numerous individuals. Phenotyping for Fusarium head blight (FHB) reactions is demanding in time and labor, and therefore expensive. Kaeppler et al. (1993) proposed a method for mapping qualitative traits, based on a binomial probability equation, which requires the genotyping and phenotyping of fewer individuals. The theory of this method implies that the probability of having a marker linked to the allele introgressed from the donor parent is a function of the genetic distance and the number of crosses or selfs used to derive the lines. According to this method genetic mapping could potentially be achieved with a minimum of three genotypes: donor parent, recurrent parent, and one NIL. Nevertheless, statistical power increases when more NIL are mapped. This method can potentially be employed to identify molecular markers associated with QTL in breeding lines with known pedigrees. The advantages would be: (1) fewer individuals need to be genotyped for marker loci as compared with mapping populations, (2) the power of detecting QTL regions associated with a trait is much greater than that with mapping populations, (3) the material used have been extensively screened, precluding the need for additional phenotyping, and (4) the genotypes having the QTL of interest and linked markers are advanced breeding lines and part of a cultivar development program.

FHB has caused severe yield and quality losses of wheat during the last decade. Estimation of losses, during 1993 to 1997, for North Dakota alone surpassed three billion dollars (McMullen et al. 1997; Windels 2000). FHB epidemics are unpredictable since they depend on disease-conducive environmental conditions. Agronomic and chemical controls are generally considered impractical or ineffective (Bai and Shaner 1994). Hence, incorporation of genetic resistance, which is the

most efficacious and economical strategy, is the top priority to reduce losses caused by this disease. However, breeding for resistant cultivars is difficult given the complexity of FHB resistance, the need to screen at host plant maturity, and the large environmental effects on disease expression. Several types of resistance have been reported (Schroeder and Christensen 1963; Wang and Miller 1988; Mesterhazy 1995); among these, Type II (spread within the infected spike) has been primarily used for screenings under controlled conditions (Stack 1989; Bai and Shaner 1994). Sumai 3, a Chinese genotype known as having Type II resistance (Wang and Miller 1988; Bai and Shaner 1994; Yang 1994), has been the main source of resistance to FHB for U.S. wheat breeding programs. However, Sumai 3 itself is unadapted to the U.S. Northern Great Plains, and it is susceptible to other diseases. Genetic studies indicate that FHB resistance is quantitatively inherited (Bai et al. 1989; Snidjers 1990; Van Ginkel et al. 1996). Two to three genes controlling FHB resistance in Sumai 3 have been reported (Van Ginkel et al. 1996; Yao et al. 1997), and one to four QTL that explain up to 63% of the variation in FHB resistance have been identified in four populations, each having Sumai 3 or a derivative as one parent (Anderson et al. 2001; Kolb et al. 2001; Buerstmayr et al. 2002). Differences among researchers reporting the number of genes conditioning resistance in Sumai 3 may be due to some heterogeneity in this genotype or the background into which it was crossed (Waldron et al. 1999). The identification of molecular markers linked to QTL coding for resistance will be useful in the case of a complex disease such as FHB where, besides the time-consuming and expensive screening, there are always susceptible genotypes underscored (escapes). Molecular markers can help identify plants carrying one or more QTL simultaneously, helping to mitigate the cumbersome disease screening presently necessary in early generations.

Once linkage is established between a marker and a QTL, this QTL can be introgressed into germplasm using marker-assisted introgression and selection. A strategy, proposed by Tanksley and Nelson (1996), integrates QTL mapping and analysis with cultivar development while taking advantage of the genetic variation present in unadapted germplasm. Furthermore, QTL for resistance from different sources can be traced and incorporated in a single genotype. Yoshimura et al. (1995) proved the feasibility of accumulating resistance factors using molecular markers by pyramiding five bacterial blight resistance genes in a rice (*Oryza sativa* L.) genotype.

Molecular markers associated with FHB resistance have previously been reported (Anderson et al. 1998a, b, 2001; Bai et al. 1999; Waldron et al. 1999; Buerstmayr et al. 2002). Two QTL were found, on the basis of restriction fragment length polymorphism (RFLP) analysis of RILs derived from the cross Sumai 3/Stoa. The major QTL on chromosome 3BS (*QFhs.ndsu-3B*), derived from Sumai 3, explained 15.4% of the variation (Waldron et al. 1999). A multiple regression model consisting of three QTL explained 29.5% of the variation. Bai et al. (1999), using A (amplified)-FLP markers, tentatively mapped a major FHB resistance QTL later localized on 3BS using a set of RILs derived from Ning 7840 (Kolb et al. 2001). Ning 7840 is a FHB-resistant Chinese cultivar derived from Sumai 3.

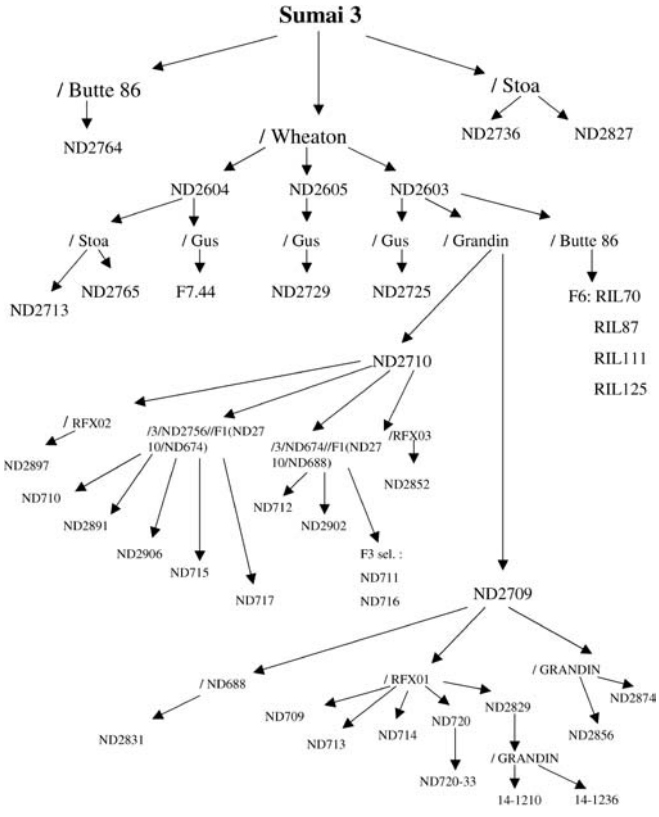

Fig. 1 Lineage of North Dakota hard red spring wheat lines

The objective of the investigation reported here was to expand on the theory of Kaeppler et al. (1993) – proposed for mapping qualitative traits – to identify microsatellite markers associated with QTL determining resistance to FHB. Resistance was inherited from Sumai 3 and present in related North Dakota adapted hard red spring wheat (HRSW) breeding lines.

Materials and methods

Thirty-six HRSW advanced lines, obtained by several cycles of crossing to North Dakota adapted genotypes and deriving their FHB resistance from Sumai 3, were used (Figs. 1, 2). Lines had previously been evaluated over five seasons of greenhouse and field testing for Type II resistance by single-floret inoculation using methods described by Stack (1989) and Stack et al. (2002). FHB disease severity was scored using a 0 to 100% scale (Stack and McMullen 1995). The advanced lines showed a wide range of variability for FHB resistance.

To accelerate the identification of markers linked to QTL for resistance, we initially screened parental genotypes and two bulked DNA samples representing resistant and susceptible lines. One bulk consisted of pooled DNA from seven lines consistently

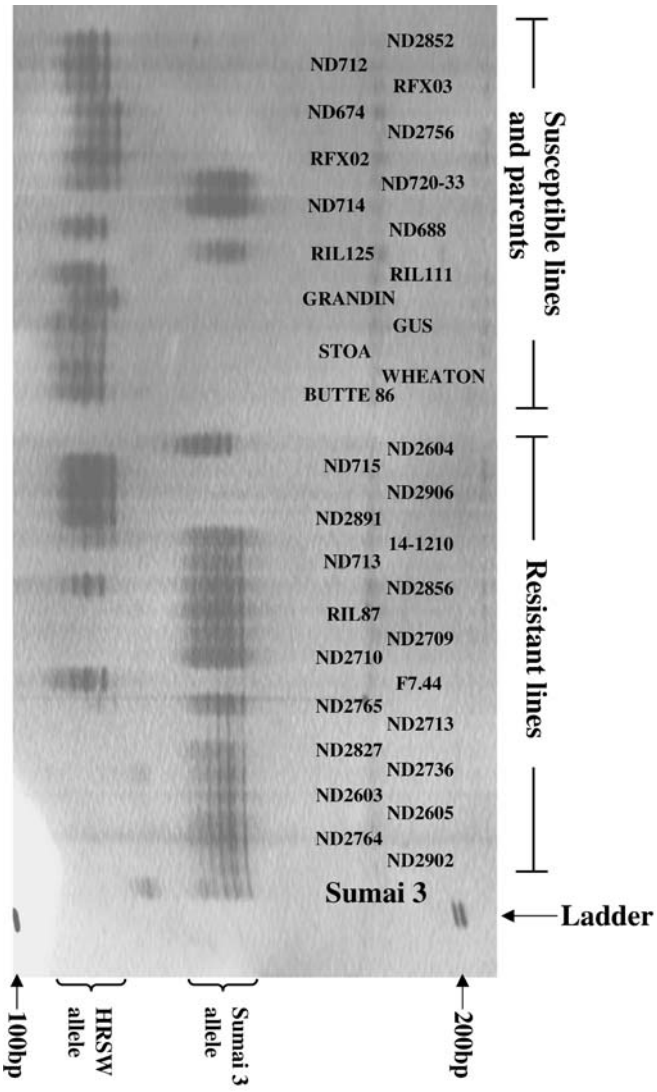

Fig. 2 DNA bands from amplification of North Dakota hard red spring wheat lines with SSR primer Gwm533

exhibiting resistance to FHB, and the other consisted of seven susceptible lines. Primers that showed polymorphism between Sumai 3 and other parents, as well as between the two bulks, were used to screen all lines.

Parents and bulks were screened for polymorphisms with 152 microsatellites markers (Röder et al. 1998). For primer selection, priority was given to those located in chromosomes where putative QTL for resistance had previously been reported (Table 1). Polymerase chain reaction products were separated by electrophoresis on 6% denaturing polyacrylamide sequencing gels. Visualization of bands was achieved by the DNA silver staining system (Promega, Madison, Wis.) according to the protocols by Bassam et al. (1991).

The probability (P) of having a donor parent allele for resistance linked to a given marker was calculated by the following equation (Kaeppler et al. 1993);

$$P = \mathrm{R!/DP!\ NoDP!}\left((0.5)^{b+1}\right)^{\mathrm{DP}}\left(1-(0.5)^{b+1}\right)^{\mathrm{noDP}}$$

where:

R = number of resistant lines,
DP = number of resistant lines with donor parent allele (from Sumai 3),
NoDP = number of resistant lines without donor parent allele,
b = number of backcrosses (even though they are not true-backcrosses, they were considered as backcrosses to a non-donor parent for the loci of interest).

Type I error rate was calculated according to the equation (Weir 1990);

$$\alpha_m = 1 - 10^{[(\log(1-\alpha_e))/n]}$$

where:

α_m = individual test error rate,
α_e = experiment error rate,
n = number of markers used.

Results and discussion

Analysis of 36 parental and derived HRSW genotypes (Fig. 1) with 152 microsatellites primer pairs (Table 1) revealed two loci significantly associated with QTL for FHB resistance. These loci were *Xgwm533* and *Xgwm274*.

Probability for locus *Xgwm533–3B*

Using the data from over five seasons of greenhouse and field testing, 19 advanced lines were phenotypically scored as FHB resistant (Fig. 2). Screening with microsatellite locus *Xgwm533*, located on chromosomes 3B (Röder et al. 1998), established 15 of the 19 FHB resistant lines as having a band at about 140 bp derived from the Sumai 3 parent (Fig. 2). According to the binomial

Table 1 Summary of microsatellite loci used for genotyping and their chromosome location according to Röder et al. (1998)

Chromosome	Microsatellite locus(*Xgwm*)
1B	11, 18, 33, 124, 131, 140, 153, 259, 264, 273, 274, 403, 413, 498, 550, 582
2B	47, 55, 120, 148, 191, 210, 257, 319, 374, 388, 429, 501, 526, 630
3B	72, 77, 108, 112, 114, 131, 181, 247, 264, 274, 284, 285, 299, 340, 376, 389, 493, 533, 547, 566
4B	6, 66, 107, 113, 149, 165, 251, 368, 495, 513, 538
5B	66, 67, 68, 159, 191, 213, 234, 335, 371, 408, 443, 499, 540, 544, 554, 604, 639
6B	70, 88, 132, 133, 191, 193, 219, 361, 508, 518, 613, 626, 644
7B	16, 43, 46, 68, 112, 146, 274, 297, 302, 333, 344, 400, 537, 569, 573, 577, 611, 644
2A	10, 47, 122, 296, 311, 425, 448, 497, 515, 636
3A	2, 5, 30, 32, 155, 162, 369, 391, 480, 574, 666
4A	4, 160, 165, 397, 601, 610
5A	126, 129, 154, 156, 179, 186, 205, 291, 293, 304, 410, 415, 595, 617, 639, 666

Table 2 Mean FHB severity for ND advanced lines carrying QTL for resistance and linked to two (*Xgwm533* and *Xgwm274*), one (*Xgwm533*), and none of the microsatellite loci

QTL linked to locus	Number of lines	Mean FHB severity (%)	$R^2 \times 100$	*P* value
Xgwm533 + Xgwm274	10	7.5	31	<0.001
Xgwm533	15	8.7	30	<0.001
None	8	21.7		

probability equation, the probability of this occurring by chance is:

$$P = 19!/15!4! \left((0.5)^{b+1}\right)^{15} \left(1-(0.5)^{b+1}\right)^{4} = 2.6 \times 10^{-15}$$

where b is equal to the number of backcrosses (from 0 to 4). For lines with no backcrosses, calculations were based on the number of selfs (Kaeppler et al. 1993). The size of chromosome 3B in wheat is estimated to be about 260 cM (Nelson et al. 1995a, b). Overall, 20 microsatellite markers were used to screen this chromosome (see Röder et al. 1998 for map location). Therefore, the approximate average distance between the markers is about 13 cM (260 cM/20 markers). Thus, the significance level calculated according to the Weir (1990) formula for a situation where 20 markers are approximately spaced 13 cM is:

$$P(\leq 0.05) = 0.0026; \quad P(\leq 0.01) = 0.0005.$$

Then, the probability that the association between marker and resistance-donor parent allele has occurred by chance is 2.6 x 10^{-15}, a highly unlikely situation. Locus *Xgwm533* is linked to the FHB resistance QTL derived from Sumai 3 and is carried along in many of the advanced HRSW breeding lines. This locus can effectively be used to select future HRSW lines derived from any of these advanced materials or any lines carrying the FHB resistance QTL coming from Sumai 3.

Probability for locus *Xgwm274–3B*

Of the 19 advanced lines scored resistant, 10 showed a band at about 160 bp derived from Sumai 3. This marker (*Xgwm274*) has been placed on chromosomes 7B and 1B (Röder et al. 1998). However, our marker analysis using wheat aneuploid and substitution lines places the 160-bp band to chromosome 3B. According to the binomial probability equation, the probability of ten lines having this locus by chance is:

$$P = 19!/10!9! \left((0.5)^{b+1}\right)^{10} \left(1-(0.5)^{b+1}\right)^{9} = 5 \times 10^{-7}.$$

As per calculations for *Xgwm533-3B*, the significance level for a situation where 20 markers are spaced approximately 13 cM apart is:

$$P(\leq 0.05) = 0.0026; \quad P(\leq 0.01) = 0.0005.$$

Then, the probability that the association between marker and resistance-donor parent allele has occurred by chance is 5 x 10^{-7}. The *Xgwm274-3B* is either linked to a minor QTL on the same chromosome or likely located farther from the QTL on 3BS than *Xgwm533-3B*, resulting in more recombinants (i.e. 15/19 vs. 10/19 resistant lines having the Sumai 3 allele).

Table 2 shows the mean FHB severity of North Dakota advanced lines carrying markers linked to loci *Xgwm533* and *Xgwm274*. Another indication that *Xgwm533* is closer to the QTL on 3BS than *Xgwm274* is that it explains the majority of the reduction in mean FHB severity (%). Only two lines carried *Xgwm274* alone; for that reason the statistical analysis for that single locus was not carried out. Nevertheless, *Xgwm274* is either linked to a minor QTL as compared to *Xgwm533* or is farther away from the locus.

A QTL region for FHB resistance on chromosome 3B was first reported by Waldron et al. (1999). This QTL designated as *QFhs.ndsu-3B*, explained 15.4% of the variation for resistance in a Sumai 3/Stoa recombinant inbred population. An AFLP marker explaining 17.6% of the variation was reported by Anderson et al. (1999) for the same region and population. The microsatellite locus *Xgwm533* also was mapped by Anderson et al. (1999) on the 3B region, but using a different population, one derived from the cross ND2603/Butte 86. The advanced line ND2603 is derived from Sumai 3; therefore, we can infer that the QTL conferring resistance has been inherited from Sumai 3. The locus *Xgwm533* has been reported by Anderson et al. (1999) as the one which explains the highest proportion of variation (24.6%) in these populations. More recently, Anderson et al. (2001) reported this QTL on 3BS as explaining 41.6% and 24.8% of the FHB variation in the populations derived from Sumai 3/Stoa and ND2603/Butte 86, respectively. Another study utilizing a large double haploid population and deriving its resistance from Sumai 3 indicated that this region explained up to 60% of variation for Type II resistance (Buerstmayr et al. 2002). A major QTL for FHB resistance, which explained 53% of the variation and originally mapped on chromosome 7B (Bai et al. 1999), has been re-assigned to chromosome 3B (Kolb et al. 2001). AFLP markers were used to identify this major QTL in a population derived from Ning 7840/Clark. Ning 7840 is a Chinese cultivar also derived from Sumai 3.

The results reported here are in agreement with previous studies reporting that *Xgwm533* is linked to QTL for FHB resistance. The method used in our study offers an efficient approach to identifying QTL by taking advantage of the available germplasm. In this study, advanced lines

obtained for breeding purposes were used to avoid the expensive and time-consuming task of developing specific populations designed for mapping. Additionally, the statistical power of detecting major QTL regions using the procedure described here is much greater and requires sampling fewer lines than using RILs or double haploid populations. Finally, the fact that the QTL regions are identified in adapted lines means these lines can serve as valuable sources of resistance parents in breeding programs. Plans are to continue the evaluation of microsatellite markers and to use allele-specific markers for *Xgwm533* and *Xgwm274* to assist selection in newer hexaploid breeding lines. These markers are also being employed in the selection of Sumai 3-derived durum breeding lines.

Acknowledgements We would like to thank Dr. José González-Hernández, Dr. Jennifer Mitchell-Fetch, Mary Johnshoy, Jana Hansen, and Justin Hegstad for all their assistance in making this paper possible. We would also like to thank Drs. McClean and Carena for their thoughtful review and suggestions in improving this manuscript. We gratefully acknowledge grant support from the USDA-ARS-U.S. Wheat and Barley Scab Initiative (59-0790-9-048) to S.F.K. and (59-0790-9-028) to W.A.B. and the North Dakota Wheat Commission.

References

Anderson JA, Waldron BL, Moreno-Sevilla B, Stack RW, Frohberg RC (1998a) DNA markers for Fusarium head blight QTL in two wheat populations. In: Hart P et al. (eds) 1998 National Fusarium Head Blight Forum. University Printing, East Lansing, pp 57–59

Anderson JA, Waldron BL, Moreno-Sevilla B, Stack RW, Frohberg RC (1998b) Detection of Fusarium head blight resistance QTL in wheat using AFLPs and RFLPs. In: Slinkard AE (ed) 9th Int Wheat Genetics Symp, vol 1. University Extension Press, Saskatoon, Canada, pp 135–137

Anderson JA, Waldron BL, Stack RW, Frohberg RC (1999) Update on DNA markers for Fusarium head blight resistance QTL in two wheat populations. In: Wagester JA et al. (eds) 1999 National Fusarium Head Blight Forum. University Printing, East Lansing, pp 19–21

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Mitchell Fetch J, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTL in two wheat populations. Theor Appl Genet 102:1164–1168

Bai GH, Shaner G (1994) Scab of wheat: prospects for control. Plant Dis 78:760–766

Bai GH, Xiao QP, Mei JF (1989) Studies on the inheritance of scab resistance in six wheat varieties. Acta Agric Shanghai 5:17–23

Bai GH, Kolb FL, Shaner G, Domier LL (1999) Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89:343–348

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80–83

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). Theor Appl Genet 104:84–91

Kaeppler SM, Phillips RL, Kim TS (1993) Use of near-isogenic lines derived by backcrossing or selfing to map qualitative traits. Theor Appl Genet 87:233–237

Kolb FL, Bai GH, Muehlbauer GJ, Anderson JA, Smith KP, Fedak G (2001) Host plant resistance genes for Fusarium head blight: mapping and manipulation with molecular markers. Crop Sci 41:611–619

McMullen MP, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81:1340–1348

Mesterhazy A (1995) Types and components of resistance to Fusarium head blight of wheat. Plant Breed 114:377–386

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Negre S, Bernard M, Leroy P (1995a) Molecular mapping of wheat. Homoeologous group 3. Genome 38:525–533

Nelson JC, Sorrells ME, Van Deynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA (1995b) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141:721–731

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53:831–838

Snijders CHA (1990) The inheritance of resistance to headblight caused by *Fusarium culmorum* in winter wheat. Euphytica 50:9–17

Stack RW (1989) Comparison of inoculum potential of ascospores and conidia of *Gibberella zeae*. Can J Plant Pathol 11:137–142

Stack RW, McMullen MP (1995) A visual scale to estimate severity of Fusarium Head Blight in Wheat. North Dakota State Univ Ext Serv, Fargo

Stack RW, Elias EM, Mitchell-Fetch J, Miller JD, Joppa LR (2002) Fusarium Head Bligh reaction of Langdon durum-*Triticum dicoccoides* chromosome substitution lines. Crop Sci 42:637–642

Tanksley SD, Nelson JC (1996) Advanced backcross Qtls analysis: a method for the simulation discovery and transfer of valuable Qtls from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

Van Ginkel M, Schaar MVD, Zhuping Y (1996) Inheritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Dis 80:863–867

Waldron BL, Moreno-Sevilla B, Andersonm JA, Stack RW, Frohberg RC (1999) RFLP mapping of QTL for Fusarium head blight resistance in wheat. Crop Sci 39:805–811

Wang Y-Z, Miller JD (1988) Screening techniques and sources of resistance to Fusarium head blight. In: Klatt AR (ed) Wheat production constraints in tropical environments. Proc Int Conf. CIMMYT, Mexico, D.F. pp 239–250

Weir BS (1990) Genetic data analysis. Sinauer Assoc, Sunderland [cited by Kaeppler et al. (1993) Theor Appl Genet 87:233–237]

Windels C (2000) Economic and social impacts of Fusarium head blight: changing farms and rural communities in the Northern Great Plains. Phytopathology 90:17–21

Yang Z (1994) Breeding for resistance to Fusarium head blight of wheat in the mid to lower Yangtze river valley of China. Wheat Special Report no. 27, CIMMYT, Mexico, D.F.

Yao JB, Ge YF, Wang S-W, Yao G-C, Zhou C-F, Qian C-M (1997) Chromosomal location of genes for scab resistance in wheat cultivar Sumai 3. Acta Agron Sin 23:450–453

Yoshimura S, Yoshimura A, Iwata N, McCouch SR, Abenes ML, Baroidan MR, Mew TW, Nelson RJ (1995) Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. In: Molecular breeding, vol 4. New strategies in plant improvement, pp 375–387