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RAPD polymorphisms in spring wheat cultivars and lines with different level of Fusarium resistance

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Abstract Random amplified polymorphic DNA (RAPD) markers have been used to characterize the genetic diversity among 35 spring wheat cultivars and lines with different levels of *Fusarium* resistance. The objectives of this study were to determine RAPD-based genetic similarity between accessions and to derive associations between *Fusarium* head blight (FHB) and RAPD markers. Two bulked DNA from either highly resistant lines or susceptible lines were used to screen polymorphic primers. Out of 160 screened primers, 17 primers generated reproducible and polymorphic fragments. Genetic similarity calculated from the RAPD data ranged from 0.64 to 0.98. A dendrogram was prepared on the basis of a similarity matrix using the UPGMA algorithm, which corresponded well with the results of principal component analysis and separated the 35 genotypes into two groups. Association analysis between RAPD markers and the FHB index detected three RAPD markers, $H19_{1000}$, $F2_{500}$ and $B1_{2400}$, significantly associated with FHB-resistant genotypes. These results suggest that a collection of unrelated genotypes can be used to identify markers linked to an agronomically important quantitative trait like FHB. These markers will be useful for marker-assistant breeding and can be used as candidate markers for further gene mapping and cloning.

Keywords DNA polymorphism · *Triticum aestivum* · Genetic diversity · *Fusarium* head blight · Cluster analysis

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Introduction

Genetic distance within cultivated crops provides a measure of the average genetic divergence between cultivars. Information about genetic relationships among accessions within species has been shown to be useful for the selection of parents for hybridization (Frei et al. 1986) and for the prediction of progeny performance (Hallauer and Miranda 1988). Knowledge about the relatedness of parents in a breeding program could also be used to avoid the possibility of elite germplasm becoming too genetically uniform, thereby endangering long-term selection gains (Messmer et al. 1993), and to conserve genetic resources.

Different methods have been used to assess the diversity of plant breeding materials. This information can be obtained by studying pedigrees, morphological traits, isozyme analysis and DNA analysis (for example, Cox et al. 1985; Shamsuddin 1985; Autrique et al. 1996). DNA markers are most suitable for genetic diversity estimates (Plaschke et al. 1995; Kim and Ward 1997; Davila et al. 1998), however the extent of their usefulness may depend on the nature of the marker, the number involved, genome coverage and the population under investigation. Random-amplified polymorphic DNA (RAPD) analysis (Williams et al. 1990) is based on the amplification of DNA fragments with the polymerase chain reaction (PCR) starting from primers with arbitrary sequences. This technique is considerably faster and simpler than some other molecular techniques. RAPD markers have been used to examine both interspecific and intraspecific variation in a number of plant species (Kazan et al. 1993; Brummer et al. 1995; Sun et al. 1997; Bai et al. 1998; Fu et al. 2002).

Fungi of the group *Fusarium* spp. have long been recognized as pathogens on many plant species. Wheat and other small-grain cereals may be attacked by *Fusarium* spp. on different organs, but the infestation of the ear appears to be most critical, leading to *Fusarium* head blight (FHB), also known as *Fusarium* ear blight (FEB), pink mold or scab. *F. graminearum* is considered to be eco-

nomically the most important *Fusarium* species in Canada because of the impact it has on yield and grain quality, its ability to produce several different toxins and its abundance in eastern Canada and the eastern prairies.

Fusarium diseases affect the growth and yield of wheat and have been estimated in some years to have caused losses approaching a billion dollars to Canadian wheat farmers. FBH of wheat also affects grain quality by reducing kernel weight and size, with infected wheat kernels often shrivelling and becoming discolored. Milling and baking quality are affected by degrading starch and proteins (Bechtel et al. 1985). The most serious threat associated with FHB is the possible accumulation of mycotoxins produced by these fungi in the kernels (Chelkowski 1991). These compounds are hazardous to humans and animals (Prelusky et al. 1994).

The risk of an FHB epidemic is high when the natural inoculum is abundant (e.g. conidia or ascospores on crop debris or the soil surface) during warm and humid weather at flowering. Changes in crop management practices (minimum or reduced tillage), changes in rainfall patterns and a low resistance level among current cultivars are considered to be the principal causes for the severe FHB epidemics that have occurred in parts of the USA and Canada since 1993 (Dill-Macky and Jones 1997; McMullen et al. 1997).

The cultivation of genetically resistant cultivars is the most cost-effective method to control the disease, and many breeders have realized the need for incorporating FHB resistance into their breeding material. Genetic variation for resistance to FHB is well-documented in wheat and its relatives (Mesterhazy 1983, 1993; Snijders 1990; Wilcoxson et al. 1992; Lemmens et al. 1993; Buerstmayr et al. 1996a, b), and it is now generally agreed that FHB resistance is governed by an oligogenic to polygenic system. Singh et al. (1995) estimated at least three resistance genes in the Brazilian cultivar Frontana, while in cv. Ning 7840, two (Van Ginkel et al. 1996) and three (Bai 1995) major resistance genes have been estimated. Several wheat chromosomes appear to carry FHB resistance genes (Buerstmayr et al. 1999a). Different resistant sources could possess different resistance genes, which could be combined in improved lines (Van Ginkel et al. 1996; Buerstmayr et al. 1999b).

The objectives of the study reported here were to estimate the genetic diversity among 35 spring wheat varieties and breeding lines with different levels of *Fusarium* resistance used at the Crops and Livestock Research Centre and to derive associations between FHB and RAPD markers. Such information will be useful in choosing parents in breeding for resistance to FHB and for candidate gene mapping.

Materials and methods

Plant materials and extraction of DNA

Thirty-five spring wheat varieties/lines varying in their reaction to *Fusarium* (Table 1) that are presently being used in the spring wheat breeding program at the Crop and Livestock Centre, Charlottetown, Prince Edward Island were used.

Leaf tissues samples were collected from these lines, frozen in liquid nitrogen and kept at –80 °C. DNA was extracted according to the method described by Junghans and Metzlaff (1990).

Field experiments for evaluation of FHB resistance

The breeding material was planted in single row plots, 50–60 cm long with 18 cm between rows and 50 cm pathways. Each line was replicated 8 times and misted for 2 min every 45 min at a rate of approximately 1000 l/ha. Plots were inoculated with a *Fusarium graminearum* spore suspension, made up of four different isolates, at 50,000 spores/ml using a tractor mounted sprayer deliering 280 l H_2O/ha. The first inoculation was made at flowering and twice more, 7 days apart. Approximately 21 days after inoculation, or when symptoms of FHB was well developed, two ratings of each plot were taken in the field. The first score represented the percentage of heads with symptoms of FHB (0–10, 0 = no head with symptoms to 10 = all heads with one symptom), while the second score represented the average amount of FHB symptoms on infected heads (0–10, where 0 = no symptoms and 10 = all infected heads completely covered with symptoms of FHB). The fusarium head blight index was the product of these two ratings (0–100). As indexes were derived from several trials, they were adjusted to Roblin's index being 100.

RAPD analysis

Amplification reactions were performed based on the stardand protocol of Williams et al. (1990) with some modifications. PCR reaction mixtures of 20 µl contained 20 ng template DNA, 0.2 m*M* of each deoxynucleiotide (dATP, dCTP, dGTP and dTTP), 2.5 m*M* $MgCl_2$, 1.0 U *Taq* polymerase (Rose company, Canada), 15 pmol of a single 10-mer primer purchased from Operon Technologies (Alameda, Calif.). Amplification was performed in DNA Thermocycler (Perkin Elmer, Foster City, Calif.). The PCR program consisted of 30 cycles of denaturation at 94 °C for 1 min, annealing at 36 °C for 1 min and extension at 72 °C for 2 min. The final cycle had a 3-min extension step at 72 °C. The PCR fragments were electrophoresed through 1.2% agarose gels, stained with 0.5 µg/ml ethidium bromide and visualized with ultraviolet light.

Data analysis

Data were scored as 1 for the presence and 0 for the absence of a DNA band for each genotype. The data matrix was entered into the NTSYS program (Rohlf 1993) and analyzed using the Qualitative routine to generate Jaccard's similarity coefficients. Similarity coefficients were used to construct a dendrogram using the UPGMA (unweighted pair group method with arithmetic average) and the SHAN (sequential, hierarchical, and nested clustering) routine in the NTSYS program. A principal coordinate analysis (PCA) was conducted with the same program using the DCENTER and EIGEN procedures. This multivariate approach was chosen to complement the cluster analysis information, because cluster analysis is more sensitive to closely related individuals, whereas PCA is more informative regarding distances among major groups (Hauser and Crovello 1982). The RAPD marker nomenclature used by Michelmore et al. (1991) was employed in describing RAPD loci.

Table 1 *Fusarium* head blight index of 35 spring wheat cultivars/lines used in this study

Number	Variety/line	Origin	*Fusarium* head blight index relative to Roblin = 100[a]
1	HC 540	China	R[b]
2	NING 68331	China	R
3	Wong Ju	China	R
4	H 281	China	R
5	Sumai-3	China	24.1
6	AW 495	AAFC-Charlottetown	17.8
7	29AA28	Coop Federee-Ste-Rosalie, Quebec	24.2
8	AC Drummond	AAFC-Ste-Foy, Quebec	24.2
9	Nyu Bay	China	28.4
10	AW 478	AAFC-Charlottetown	38.5
11	AW 499	AAFC-Charlottetown	28.3
12	95-51-10	AAFC-Charlottetown	118.7
13	95-38-34	AAFC-Charlottetown	119.2
14	96-12-10	AAFC-Charlottetown	121.8
15	AC Hartland	AAFC-Charlottetown	44.9
16	AW 373	AAFC-Charlottetown	45.5
17	QW 625.25	AAFC-Ste-Foy, Quebec	47.0
18	QW 628.5	AAFC-Ste-Foy, Quebec	37.4
19	AW 466	AAFC-Charlottetown	47.5
20	AC Helena	AAFC-Charlottetown	52.5
21	Belvedere	AAFC-Charlottetown	58.9
22	Grandin	University of Minnesota	58.0
23	Max	Germany	55.9
24	972-B1	Germany	63.9
25	972-B5	Germany	63.9
26	AC Norboro	AAFC-Charlottetown	66.1
27	AW 492	AAFC-Charlottetown	47.3
28	AW 491	AAFC-Charlottetown	52.4
29	Celtic	U.S.A.	76.0
30	Pacific	AAFC-Winnipeg	81.2
31	Glenlea	University of Manitoba	57.9
32	AW 493	AAFC-Charlottetown	58.9
33	AW 498	AAFC-Charlottetown	58.9
34	AC Walton	AAFC-Charlottetown	61.7
35	Roblin	AAFC-Winnipeg	100

[a] Index = severity incidence (0–10)×severity in infected heads (0–10), and adjusted to Roblins index being 100
[b] Resistant but no local data available

Results

DNA polymorphism

Two DNA bulks, each resistant or susceptible to *Fusarium*, were prepared by pooling the DNA from five varieties or lines representing higher resistant and higher susceptible levels. The bulked DNAs were used to screen the polymorphic and repeatable RAPD primers of a total of 160 primers from Operon Technologies screened with the two bulks, 17 primers (11%) gave polymorphic RAPD patterns. Sixteen random 10-mer primers that resulted in very clear RAPD patterns were selected to amplify fragments from the DNA temples of 35 genotypes. A total of 67 strong bands were scored, giving an average of 4.2 fragments per primer. Of the 67 fragments, 42 (63%) were polymorphic across the 35 genotypes and gave from one to seven polymorphic fragments with a mean of 2.6 polymorphic fragments per primer.

Genetic diversity

The genetic similarity matrix was produced for the RAPD data using the Jaccard algorithm (Table 2). Genetic similarity coefficients ranged from 0.64 in a pair of genotypes (Nyu Bay and 972-B1) to 0.98 in each of four pairs (between H281 and Pacific, between AW478 and 95-38-34, between AW478 and AW373 and between Celtic and Glenlea). Genetic similarity values were used for cluster analysis through UPGMA, resulting in a dendrogram (Fig. 1). The genotypes were grouped into two clusters, with cluster I containing 29 genotypes and cluster II containing six genotypes. The 29 genotypes belonging to cluster I were again grouped into two sub-clusters: sub-cluster I-2 contains one genotype, Nyu Bay; sub-cluster I-1 contains 28 genotypes. Sub-cluster I-1 was further divided into two groups, I-1-1 and I-1-2. In group I-1-1, three higher level FHB resistance genotypes were grouped into subgroup a. The 4 genotypes with higher FHB index values were clustered into subgroup b. All genotypes except Sumai-3 and AW499 in group I-1-2 have intermediate to higher FHB index values. Five higher FHB-susceptible genotypes were assigned to sub-

Table 2 Matrix of pair-wise genetic similarity between the 35 spring wheat cultivars or lines based on Jaccard's coefficients

Number[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	1.00																																		
2	0.91	1.00																																	
3	0.94	0.92	1.00																																
4	0.91	0.92	0.89	1.00																															
5	0.87	0.88	0.84	0.84	1.00																														
6	0.81	0.75	0.78	0.79	0.71	1.00																													
7	0.89	0.83	0.84	0.91	0.79	0.75	1.00																												
8	0.82	0.79	0.78	0.84	0.73	0.89	0.83	1.00																											
9	0.86	0.91	0.91	0.81	0.89	0.70	0.77	0.69	1.00																										
10	0.92	0.86	0.86	0.89	0.92	0.79	0.87	0.80	0.88	1.00																									
11	0.90	0.88	0.87	0.91	0.87	0.85	0.89	0.89	0.86	0.95	1.00																								
12	0.87	0.85	0.81	0.81	0.90	0.78	0.82	0.78	0.86	0.92	0.93	1.00																							
13	0.93	0.87	0.88	0.87	0.93	0.76	0.85	0.76	0.89	0.98	0.92	0.93	1.00																						
14	0.90	0.91	0.84	0.94	0.84	0.79	0.92	0.83	0.80	0.91	0.93	0.87	0.89	1.00																					
15	0.90	0.88	0.89	0.95	0.81	0.78	0.95	0.83	0.80	0.88	0.90	0.83	0.85	0.96	1.00																				
16	0.90	0.85	0.84	0.87	0.90	0.77	0.89	0.78	0.86	0.98	0.93	0.93	0.96	0.93	0.90	1.00																			
17	0.97	0.95	0.95	0.94	0.87	0.78	0.89	0.82	0.86	0.89	0.90	0.84	0.89	0.90	0.92	0.87	1.00																		
18	0.89	0.93	0.83	0.89	0.86	0.82	0.84	0.86	0.82	0.87	0.95	0.92	0.88	0.92	0.87	0.85	0.89	1.00																	
19	0.85	0.83	0.82	0.85	0.82	0.79	0.94	0.87	0.81	0.90	0.95	0.92	0.87	0.91	0.90	0.91	0.85	0.90	1.00																
20	0.91	0.86	0.85	0.92	0.88	0.81	0.87	0.81	0.84	0.96	0.91	0.88	0.94	0.91	0.90	0.95	0.88	0.87	0.86	1.00															
21	0.77	0.78	0.77	0.83	0.73	0.91	0.74	0.89	0.73	0.75	0.84	0.75	0.71	0.79	0.80	0.74	0.81	0.81	0.79	0.77	1.00														
22	0.93	0.91	0.87	0.91	0.87	0.79	0.89	0.80	0.83	0.91	0.90	0.90	0.92	0.96	0.92	0.93	0.90	0.92	0.88	0.91	0.75	1.00													
23	0.85	0.87	0.80	0.86	0.83	0.78	0.84	0.82	0.82	0.90	0.95	0.92	0.88	0.92	0.87	0.92	0.86	0.90	0.93	0.90	0.78	0.88	1.00												
24	0.80	0.73	0.72	0.74	0.68	0.83	0.73	0.78	0.64	0.74	0.72	0.70	0.70	0.78	0.77	0.76	0.77	0.71	0.70	0.76	0.80	0.78	0.73	1.00											
25	0.84	0.81	0.81	0.87	0.77	0.91	0.82	0.88	0.76	0.86	0.85	0.78	0.83	0.86	0.85	0.84	0.85	0.81	0.79	0.88	0.91	0.82	0.81	0.87	1.00										
26	0.87	0.88	0.81	0.84	0.90	0.73	0.85	0.73	0.86	0.91	0.86	0.93	0.92	0.90	0.87	0.93	0.84	0.89	0.88	0.91	0.69	0.93	0.88	0.71	0.79	1.00									
27	0.84	0.89	0.81	0.85	0.93	0.70	0.83	0.75	0.87	0.89	0.84	0.91	0.90	0.88	0.84	0.91	0.85	0.86	0.86	0.89	0.71	0.91	0.86	0.70	0.76	0.94	1.00								
28	0.83	0.76	0.74	0.80	0.73	0.90	0.80	0.84	0.66	0.80	0.78	0.76	0.76	0.84	0.83	0.82	0.79	0.76	0.76	0.82	0.86	0.84	0.76	0.92	0.95	0.77	0.76	1.00							
29	0.86	0.91	0.83	0.86	0.94	0.71	0.80	0.76	0.88	0.91	0.89	0.89	0.92	0.89	0.84	0.89	0.86	0.91	0.84	0.91	0.72	0.89	0.88	0.67	0.81	0.92	0.91	0.73	1.00						
30	0.94	0.91	0.92	0.98	0.84	0.83	0.93	0.87	0.83	0.92	0.94	0.84	0.90	0.96	0.96	0.90	0.94	0.91	0.89	0.94	0.82	0.92	0.87	0.78	0.90	0.85	0.83	0.84	0.88	1.00					
31	0.89	0.93	0.83	0.89	0.92	0.75	0.84	0.78	0.88	0.93	0.92	0.92	0.94	0.92	0.87	0.92	0.89	0.93	0.87	0.93	0.75	0.92	0.90	0.70	0.85	0.95	0.93	0.76	0.98	0.91	1.00				
32	0.93	0.87	0.88	0.87	0.86	0.76	0.92	0.80	0.83	0.91	0.89	0.89	0.93	0.92	0.89	0.92	0.89	0.88	0.91	0.87	0.71	0.96	0.84	0.74	0.79	0.89	0.90	0.80	0.84	0.90	0.88	1.00			
33	0.92	0.90	0.87	0.94	0.82	0.77	0.92	0.81	0.80	0.87	0.89	0.86	0.89	0.96	0.95	0.89	0.93	0.91	0.87	0.90	0.77	0.96	0.87	0.76	0.84	0.89	0.86	0.83	0.88	0.96	0.91	0.92	1.00		
34	0.87	0.93	0.84	0.88	0.89	0.72	0.85	0.78	0.86	0.89	0.87	0.87	0.90	0.90	0.87	0.87	0.87	0.93	0.85	0.88	0.73	0.90	0.85	0.68	0.79	0.94	0.89	0.74	0.94	0.90	0.96	0.86	0.89	1.00	
35	0.90	0.88	0.89	0.96	0.83	0.79	0.90	0.83	0.87	0.92	0.91	0.84	0.90	0.94	0.93	0.90	0.91	0.89	0.85	0.96	0.82	0.90	0.89	0.73	0.87	0.87	0.84	0.79	0.90	0.98	0.92	0.87	0.94	0.88	1.00

[a] See Table 1 for the indentification numbers of cultivars/lines

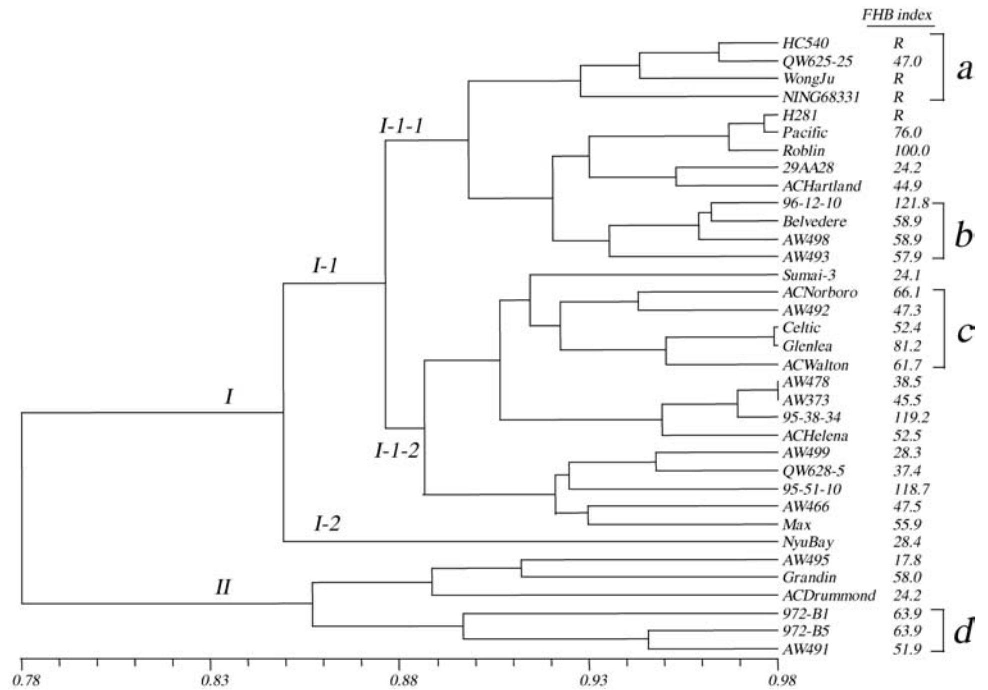

Fig. 1 UPGMA dendrogram showing genetic relationships among the 35 spring wheat genotypes used in this study. The dendrogram was constructed based on genetic similarity calculated according to Jaccard's coefficient

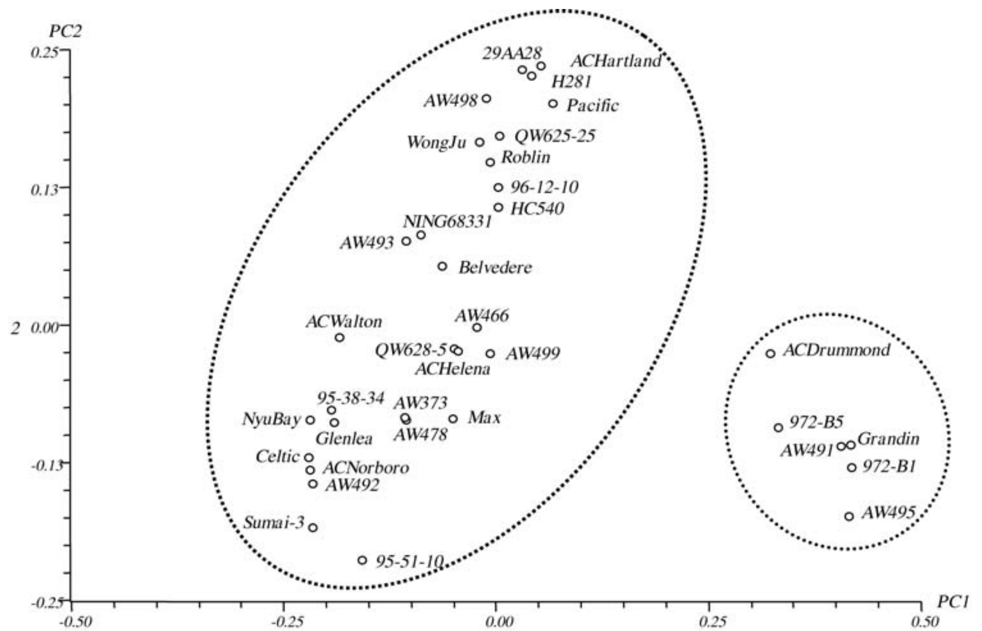

Fig. 2 Associations between 35 genotypes pn the basis of the first two principal coordinates (*PC1*, *PC2*) obtained from a principal coordinate analysis of Jaccard similarity coefficients based on RAPD data

group c. The three genotypes with higher FHB indices in cluster II were also grouped together (subgroup d).

Associations among the 35 genotypes were also examined with PCA (Figs. 2 and 3). Principal coordinates 1, 2 and 3 explained 27%, 13% and 11%, respectively, of the associations. The PCA method separated the 35 genotypes into two distinct groups by the first principal coordinate (Figs. 2 and 3). The results of PCA corresponded well to those from cluster analysis obtained through UPGMA. The six Chinese resistant genotypes of similar origin were grouped together in the third coordinate (Fig. 3).

Markers associated with FHB resistance

Table 1 lists FHB indices for 35 genotypes used in this study ranging from highly susceptible to highly resistant.

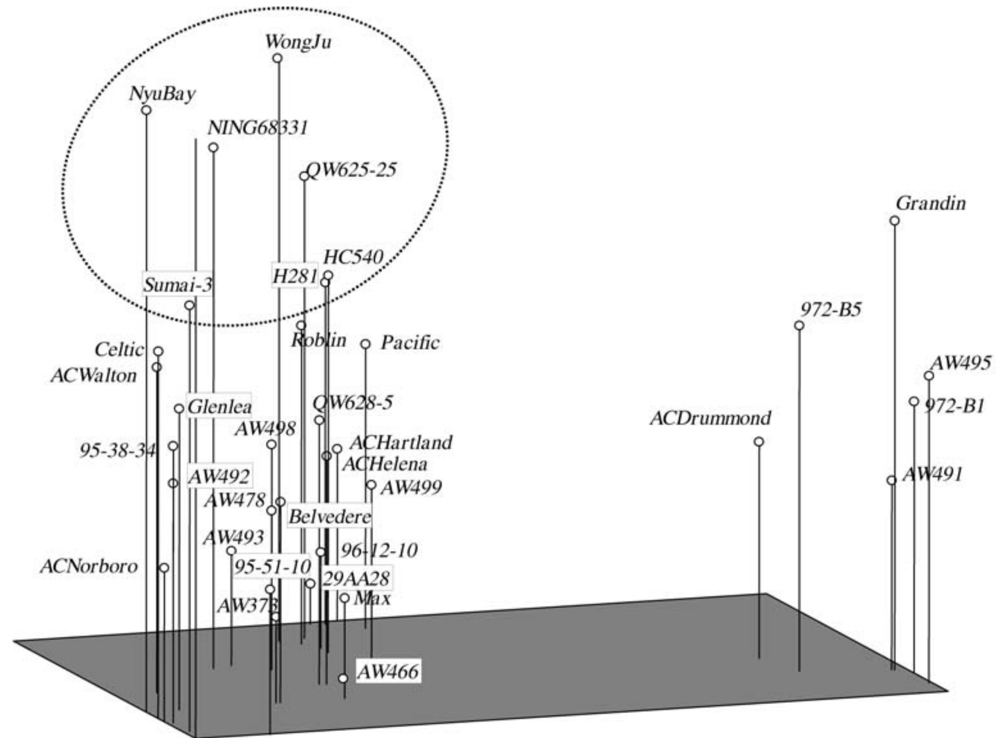

Fig. 3 Patterns of relationships among 35 spring wheat genotypes with different level of FHB index revealed by principal component analysis based on RAPD data. The third axis separated the Chinese genotypes with lower level of FHB index

Since the cluster analysis showed an association between FHB index for the genotypes and their genetic relatedness, we tested whether some RAPD marker frequencies were significantly different between genotypes with high (>60.0) versus low (<30.0) FHB index values. Three markers, $H19_{1000}$, $F2_{500}$ and $B1_{2400}$, were associated with low FHB index. A χ^2 test showed that the association was significant (Table 3).

Discussion

The level of RAPD polymorphism within wheat itself has been reported to be low (Devos and Gale 1992). Joshi and Nguyen (1993) assessed the extent of genetic diversity detected by the RAPD technique among 15 varieties of common bread wheat and found that out of 109 amplified fragments, 71 (65%) were polymorphic in these wheat cultivars. In the current study, of the 67 amplified fragments, 42 (63%) were polymorphic across the 35 genotypes, with the level of polymorphism concurring with that found by Joshi and Nguyen (1993). However, the level of RAPD polymorphism in wild wheat has been found to be higher than that in common bread wheat. Castagna et al. (1997) estimated the genetic diversity among 49 accessions of *Triticum urartu* Tum. and found that out of a total of 161 amplified products, 155 (96%) bands were polymorphic. Differences in the results obtained from various studies on levels of polymorphism can be attributed to differences among the genotypes chosen for the studies.

The genetic similarities calculated for all pairwise comparisons among the 35 spring wheat genotypes ranged from 0.64 to 0.98. In comparison, a study of 15 varieties of common bread wheat for 109 RAPD fragments found that similarities among cultivars ranged from 0.60 to 0.90 (Joshi and Nguyen 1993). The relatively low genetic diversity observed in our study may be that only the genome regions conferred to *Fusarium* resistance were tagged since the primers generated polymorphism between the resistant and susceptible bulked DNAs were used to characterize these 35 genotypes.

The results of genetic diversity based on the dendrogram derived using genetic similarity (GS) values and the PCA analysis conducted directly from binary matrix data were in agreement to a great extent (Figs. 1–3). The highest GS value of 0.98 was detected between H281 and Pacific, between AW478 and 95-38-34, between AW478 and AW373 and between Celtic and Glenlea, suggesting that the two genotypes in each of these four pairs are genetically closely related. This corresponds well with their positions in the dendrogram as well as in the PCA plot (Figs. 1 and 2). The pair of Nyu Bay from China and 972-B1 from Germany had the lowest GS value (0.64). On the basis of available information on pedigree (available pedigree data were inadequte to calculate coefficients of coancestry, *f*), AW478 and 95-38-34 were ancestrally related, possessing two common parents, Kolibri and Gamenya. AW478 and AW373 shared a common parent, Kolibri. Three genotypes, AW491, AW492 and AW493, have the same parentage (AC Voyageur/4/Gamenya/Kolibri/3/N.Bozu/2/Gamenya/

Table 3 RAPD markers whose frequencies differed significantly between the resistant (FHB index <20.0) and susceptible (FHB index >40.0) groups

Genotypes	HC 540	MING 68331	Wong Ju	H281	Sumai-3	AW 495	29AA28	AC Drummond	Nyu Bay	AW499	972-B1	972-B5	AC Norboro	Pacific	Glenlea	AC Walton	Robl	95-51-10	95-38-34	96-12-10	Marker Frequency Index <30	Index >60	Difference
FHB index	R	R	R	R	24.1	17.8	24.2	24.2	28.4	28.3	63.9	63.9	66.1	76.0	81.2	61.7	100	118.7	119.2	121.8			
Markers																							
$H19_{1000}$	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0.5*
$F2_{500}$	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0.4	0	0.4*
$B1_{2400}$	1	0	1	0	1	1	1	0	1	1	0	0	0	0	0	0	0	1	1	0	0.7	0.2	0.5*

* Significant at the 0.05 probability level

Kolibri). Cluster analysis and PCA analysis separated these three genotypes from each other. The discrepancy between pedigree and RAPD base genetic diversity estimates may be caused by selection pressure for different breeding objectives. Some genotypes, for instance, 96-12-10 and Belvedere, AW478 and AW373, 95-38-34 and AC Helena, were ancestrally related, and grouped together (Fig. 1), showing a certain degree of correlation between DNA data and pedigree. Low to moderate correlation coefficients were reported between matrices based on amplified fragment length polymorphism (AFLP) genetic diversity estimates and kinship in durum wheat (Soleimani et al. 2002), between coefficient of parentage and AFLP in hexaploid wheat (Barrett et al. 1998) and between restricted (R)FLP and pedigree data in durum wheat (Autrique et al. 1996).

Many breeders have realized the need for incorporating FHB resistance into their breeding material. Even if the disease occurs in nature only sporadically, resistant genotypes will withstand epidemic years much better than susceptible ones. However, breeding for FHB resistance is difficult for various reasons: (1) the most resistant germplasm is of exotic origin and has poor agronomic traits, (2) the inheritance is oligogenic to polygenic and (3) screening for FHB resistance is environmentally biased, tedious and expensive (Buerstmayr et al. 2002). The application of molecular markers could complement classical plant breeding. A number of successful reports on the development and application of molecular markers in improving disease resistance in wheat have been published (see reviews by Langridge and Chalmers 1998; Gupta et al. 1999), but only a few results have been published on molecular mapping of FHB resistance (Moreno-Sevilla et al. 1997; Bai et al. 1999; Waldron et al. 1999; Ban 2000; Gilbert and Tekauz 2000; Anderson et al. 2001; Buerstmayr et al. 2002). Our study detected three RAPD markers significantly associated with FHB-resistant genotypes; these will be useful for marker-assistant breeding and can be used as candidate markers for further gene mapping and cloning. Perhaps the most significant aspect of the current study was the demonstration that a collection of unrelated genotypes can be used to identify markers linked to an agronomically important quantitative trait like FHB. Typically, the identification of markers linked to target genetic loci has routinely involved the construction of genetic linkage maps from an appropriate segregating population or the identification of differences between near-isogenic lines. The current study is analogous to studies wherein the authors identified sex-specific markers in ostrich based on a collection of birds (Bello and Sanchez 1999) or characterized the loci involved in hypertension disease in humans based on statistical association analysis (Jeunemaître et al. 1992). In plants, Sun et al. (2001) identified 17 RAPD markers and five microsatellite markers significantly associated with the Ontaro Corn Heat Unit ratings of corn hybrids based on the collection of unrelated hybrids. The advantage of using a collection of individuals rather than a population to identify markers for traits is

that the markers are more likely to be applicable to a large number of breeding programs (Sun et al. 2001). Markers that come from traditional linkage studies always need to be validated by testing them with a variety of populations to determine if they are for general use in marker-assisted selection.

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