Theor Appl Genet (2005) 111: 521–529
DOI 10.1007/s00122-005-2043-3

ORIGINAL PAPER

A. Steed · E. Chandler · M. Thomsett · N. Gosman
S. Faure · P. Nicholson

Identification of type I resistance to Fusarium head blight controlled by a major gene located on chromosome 4A of *Triticum macha*

Received: 22 December 2004 / Accepted: 18 April 2005 / Published online: 20 May 2005

Abstract Using a set of 21 substitution lines of *Triticum macha* in a 'Hobbit Sib' background, it was previously demonstrated that chromosome 4A of *T. macha* carries significant resistance to Fusarium head blight. In the present study, the *T. macha* 4A resistance was further characterized in a 'Hobbit Sib' (*T. macha* 4A) single-recombinant chromosome doubled haploid (DH) population. Lines were phenotyped for disease resistance, yield components and deoxynivalenol (DON) mycotoxin content over two consecutive seasons. Both resistance to spread and resistance to initial infection were examined, and it was established that the resistance residing on *T. macha* 4A is predominantly of type I (resistance to initial infection). It was demonstrated that this type I resistance significantly lowered levels of DON accumulation in the grain and improved yield components under high disease pressure. Genotyping the DH lines using microsatellite genetic markers enabled the location of the gene(s) for resistance to be assigned to a region of the short arm of chromosome 4A, distal to microsatellite marker *Xgwm601* and co-segregating with microsatellite marker *Xgwm165* in this population.

Introduction

The predominant causal agent of Fusarium head blight (FHB) in the UK is *Fusarium culmorum*, with other important species being *Fusarium graminearum*, *Fusarium avenaceum*, *Fusarium poae* and *Microdochium nivale*. This disease is prevalent in many high-rainfall, cereal-growing regions of the world. FHB can cause large reductions in yield (Snijders and Perkowski 1990), associated with both the characteristic bleaching of ears, a symptom of penetration and spread of the pathogen through the rachis into adjacent spikelets (Kang and Buchenauer 2000) and the invasion of the kernel by the fungus, resulting in destruction of the starch granules, and storage proteins causing the familiar symptom of shrivelled 'tombstone' grain. Potentially more important are the economic losses caused by the accumulation of mycotoxins in the grain, which accrue from the reduced price paid for contaminated grain at point of sale and the costs of mycotoxin testing (Joffe 1983; Windels 2000). *F. culmorum* produces the trichothecenes, nivalenol and/or deoxynivalenol (DON), both inhibitors of protein synthesis and both with deleterious effects on animal and human health, including dermatitis, anaemia and immunosuppresion (Berek et al. 2001). The fungus is thought to use these secondary metabolites as aggressiveness/virulence factors, aiding colonization of the host (Desjardins et al. 1996; Harris et al. 1999). The control of FHB in small-grain cereals is predominantly by fungicide application. The effectiveness can be unpredictable due to the number of organisms present in the disease complex, the timing of fungicide application and the development of fungicide resistance (Parry et al. 1995). There is also a growing body of evidence that suggests complex interactions between the environment, the pathogens and some fungicides can result in elevated mycotoxin levels (Milus and Parson 1994; Simpson et al. 2001). This highlights the importance of exploiting genetic resistance to breed resistant wheat varieties, thereby reducing the need for fungicide application. Two broad types of resistance to FHB are recognized (Schroeder and Christensen 1963), resistance to initial infection (type I) and resistance to spread within the spike (type II). Most work reported to date has focused on type II resistance, as it is less difficult to characterize than type I. Further types of resistance have been postulated (reviewed by Ban 2000). However, these are not assessed in this paper. To date no resistance source has been identified which can offer complete resistance to wheat from FHB. Partial resistances have been

Communicated by D.A. Hoisington

A. Steed (✉) · E. Chandler · M. Thomsett · N. Gosman
S. Faure · P. Nicholson
John Innes Centre, Norwich Research Park,
Colney, Norwich, NR4 7UH, UK
E-mail: andrew.steed@bbsrc.ac.uk

discovered in a wide variety of hosts from Asia ('Sumai 3', 'Nobeokabozu'), South America ('Frontana', 'Encruzilhada') and Europe ('Praag 8', 'Arina') (Snijders 1990). Resistance to FHB is complex and generally appears to be oligo-/polygenic (Kolb et al. 2001). In the best-studied source of resistance, 'Sumai 3', the resistance appears to be determined by quantitative trait loci (QTL) on several chromosomes. A major QTL on chromosome 3B (*Qfhs.nsdu-3BS*) and another on 5A (*Qfhs.ifa-5A*) have been identified in 'Sumai 3' and its derivatives (Beurstmayr et al. 2003; Liu and Anderson 2003), and others have also been observed in lines derived from this source (Shen et al. 2003; Yang et al. 2003) Another source of resistance to FHB is among exotic germplasm and the wild relatives of wheat (Gilbert and Tekauz 2000; Dardis and Walsh 2003). Resistance to FHB in *Triticum macha*, a normal hexaploid land race wheat has previously been shown by Grausgruber et al. (1998) and Mentewab et al. (2000) to be polygenic in nature, with a large component of the resistance residing on chromosome 4A. The present study utilized a doubled haploid (DH) population generated from the cross between the single-chromosome intervarietal substitution line 'Hobbit Sib': *T. macha* 4A (HsTm4A) and 'Hobbit Sib'. Using this population, we have been able to characterize the resistance residing on *T. macha* 4A and how it affects disease levels, yield components and mycotoxin accumulation. We have also been able to define further the map location for the gene (small group of genes) controlling FHB resistance on chromosome 4A.

Materials and methods

Fungal material

A single aggressive DON-producing *F. culmorum* isolate (Fu 42) obtained from the John Innes Centres Facultative Pathogen Collection was used in all experiments. The isolate was cultured in flasks on a wheat and barley grain mixture at 20°C for 21 days. The conidia were washed from the grain, rinsed and adjusted to 1×10^6 conidia ml^{-1} and stored at −20°C until required. Prior to inoculation, the inoculum was amended by the addition of Tween-20 to a final concentration of 0.05% (v/v).

Plant material and inoculation

A DH (HsTm4ADH) population was generated from the cross between HsTm4A × 'Hobbit Sib'. DHs were generated following the method of Laurie and Reymonde (1991), with modifications (Gosman 2002). Disease and yield trials were conducted with 43 DH lines and the two parents in a polythene horticultural tunnel to maintain high humidity, over two seasons. Temperature in the polytunnel was slightly higher than ambient, with average daytime temperatures during June being 17°C, whereas light levels were slightly reduced because of the translucent polythene. Individual plants were grown in 1-l pots filled with John Innes No. 2 loam-based potting compost adjusted to pH 7.5 with lime. Plants were randomized in four replicated blocks, three or four plants per line in each block. Two ears per plant were spray-inoculated until run off, at mid-anthesis (GS 64, Zadoks et al. 1974) with 1×10^5 conidia ml^{-1} inoculum, in 2001 and 2002. In 2002, a single ear per plant was point-inoculated with 30 µl of 1×10^6 conidia ml^{-1} applied to a centrally located spikelet, cut to reveal the anthers in the middle floret. These methods enable evaluation for type I + type II resistance and type II resistance, respectively. Point-inoculated and non-inoculated control ears were covered for 7 days with small cellophane crossing bags to prevent contamination.

Phenotypic data collection and analysis

Visual disease symptoms were evaluated 7, 14 and 21 days post inoculation (dpi). Spray-inoculated ears were scored as percentage of the spikelets showing disease symptoms and converted to area under the disease progress curve (AUDPC). Point-inoculated ears were visually scored for the number of spikelets infected. Yield components were measured in both years. Total ear weight (2001 and 2002), mean spikelet weight and mean grain weight per spikelet (2002 only) were determined for spray-inoculated and non-inoculated control plants. These data were expressed as percentages of the non-inoculated control for each line and are referred to as relative ear weight (REW), relative spikelet weight (RSpW) and relative grain weight per spikelet (RGWSp).

DON quantification was carried out on grain samples from two blocks of a randomly selected sub-set of 22 lines from the spray-inoculated trial of 2002. In order to avoid underestimating DON levels, ears were hand-harvested and manually threshed to retain small highly infected kernels. A representative sub-sample (4 g) was milled to flour, and a 1-g sub-sample of this was extracted in water. The extract was then analysed using a competitive enzyme immunoassay kit for the quantitative analysis of DON (R-Biopharm, Germany) according to the manufacturer's instructions. Samples were diluted appropriately to permit quantification by reference to a standard curve generated from DON standards provided with the kit.

Statistical analysis

Analysis of variance was carried out on visual disease and yield data using a general linear model to partition the variance attributable to genotype and to assess year × genotype and genotype × block interactions. One-way analysis of variance incorporating Dunnett's intervals

for treatment means tests ($P < 0.05$) with the parents as standards was used to assign genotypes into ('Hobbit Sib'-like) susceptible or (HsTm4A-like) resistant groups. Two-sample *t*-tests ($P < 0.05$) were used to confirm whether there was a significant difference between the two groups. Pearson coefficients of correlation were used to analyse the relationship between trait means. All statistical analyses were undertaken using Minitab, release 13 (Minitab, USA).

Genotyping

DNA was extracted from fresh leaf tissue of all the DH lines using a modified CTAB method (Nicholson et al. 1996). The two parents, HsTm4A and 'Hobbit Sib', were screened with microsatellite primers known to be located on chromosome 4A of *T. aestivum*. Only microsatellites *Xgwm165*, *Xgwm601* and *Xgwm610* were polymorphic. Recombinant inbred lines (86) from the International Triticeae Mapping Initiative (ITMI) population derived from the cross 'Opata85' × W-7984 (Van Deynze et al. 1995) were assayed with these microsatellites. Their map locations on chromosome 4A were established by combining the results with the ITMI RFLP data set (GrainGenes, http://wheat.pw.usda.gov/index.shtml).

Due to the low level of microsatellite polymorphism between HsTm4A and 'Hobbit Sib', a sequence-specific amplified polymorphism (SSAP) technique was used to identify further markers. The technique was based on the retrotransposon DNA fingerprinting protocol of Kalender et al. (1999), using the domain-specific primer Sukkula 9900 (5′-GAT AGG GTC GCA TCT TGG GCG TGA C-3′) and a *Taq*I adaptor primer with a selective three base extension. PCR conditions were as those of Vos et al. (1995). SSAP and microsatellite markers were used to genotype the HsTm4ADH lines and define the location of the FHB resistance. Linkage maps were generated using JoinMap, version 2.0 (Stam and van Ooijen 1995). Microsatellites were obtained from an international consortium led by Agrogene, Moissy Cramayal, France, and Röder et al. (1998). PCR conditions were as described by Bryan et al. (1997), and PCR products were separated on 6% polyacrylamide and visualized by silver staining (Promega).

Results

Spray inoculation

Disease progression across the trials was lower in 2002 than in 2001. In both years disease progress in the susceptible parent 'Hobbit Sib', as measured by AUDPC, was more rapid than in the resistant parent HsTm4A (Fig. 1a, b). In both 2001 and 2002, two-sample *t*-test confirmed the parents to be significantly different from each other ($P < 0.001$), with HsTm4A being significantly

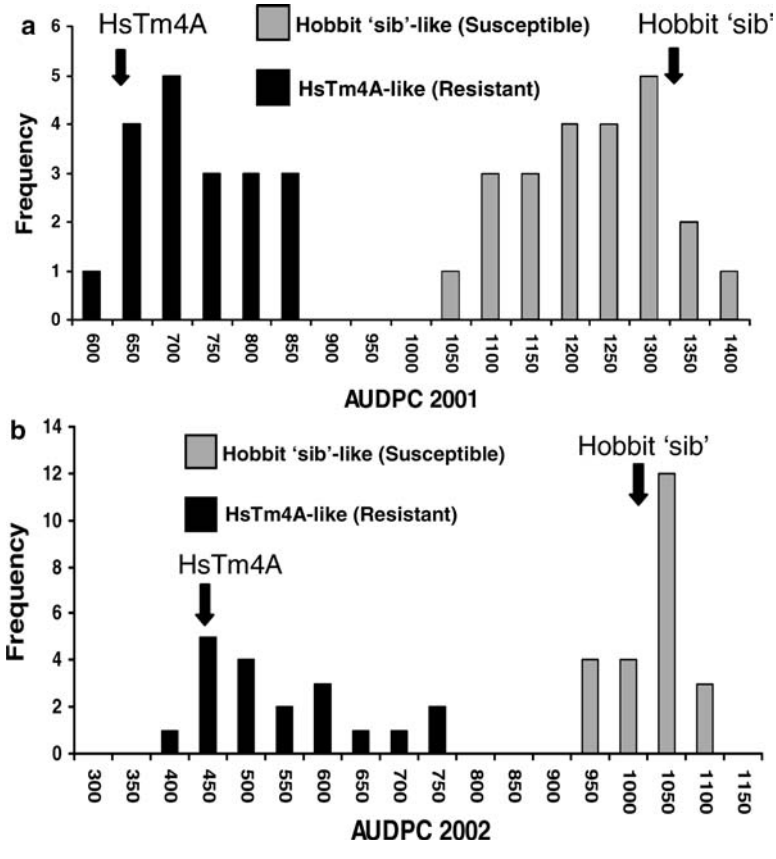

Fig. 1 Frequency distribution of 43 spray-inoculated doubled haploid (DH) lines for area under the disease progress curve (*AUDPC*) in 2001 (**a**) and 2002 (**b**). Groups were determined by Dunnet's one-way analysis of variance as 'Hobbit Sib'-like (*susceptible*) or HsTm4A-like (*resistant*). Value for parental genotypes are indicated

Table 1 Analysis of variance for area under the disease progress curve (*AUDPC*) and relative ear weight (*REW*) for *Fusarium culmorum* spray-inoculated ears in 2001 and 2002

Source	*df*	AUDPC			REW		
		MS	*F*-value	*P*	MS	*F*-value	*P*
Replications (in years)	3	90,587	2.86	0.036	3755.4	21.43	< 0.0001
Year	1	10,071,526	318.26	< 0.0001	6550.8	37.39	< 0.0001
Genotype	41	1,715,349	54.21	< 0.0001	5334.1	30.45	< 0.0001
Genotype × year	41	8,1195	2.57	< 0.0001	542.2	3.09	< 0.0001
Error	1,074	31,646			175.2		

less diseased than 'Hobbit Sib'. Analysis of variance indicated that over both years genotypic variation was significant for both AUDPC and REW. The effect of years was highly significant, which can be attributed to the lower level of disease in 2002, and there were small but significant genotype × year interactions (Table 1). A bimodal frequency distribution of AUDPC centred on the parents was observed in both years (Fig. 1a, b). Dunnett's comparison, using the 'Hobbit Sib' parent as the control was used to allocate lines into two groups, those which were 'Hobbit Sib'-like (susceptible) and those which were HsTm4A-like (resistant). This procedure allocated the same lines to the two groups in both years. In both 2001 and 2002 a two-sample *t*-test confirmed that the two groupings were significantly different ($P < 0.001$), with the 'Hobbit Sib'-like susceptible grouping having the higher level of disease. The segregation ratio of resistant to susceptible phenotypes did not differ significantly from the 1:1 distribution expected for a single gene, $\chi^2 = 0.395$.

Although there was a small genotype × year interaction the genotypic correlation coefficient of AUDPC for paired means between years was $r = 0.854$, 1 *df*, $P < 0.001$.

A bimodal distribution was also found for total REW to the control in both years (Fig. 2a, b). Dunnett's comparison, using the 'Hobbit Sib' parent as the control, was used to allocate lines into two groups. The procedure allocated the same lines to each grouping in both years. The lines within each group were the same as those seen with AUDPC, with all lines having the significantly higher REW also having significantly more resistance than 'Hobbit Sib'. In both years a two-sample *t*-test confirmed that the two groupings for REW were significantly different ($P < 0.001$). The results for REW were strongly correlated between years ($r = 0.804$, 1 *df*, $P < 0.001$). AUDPC and REW were strongly correlated in both years $r = -0.913$ and $r = -0.962$, 1 *df*, $P < 0.001$ for 2001 and 2002, respectively (Table 2). In 2002, the yield components for spray-inoculated ears were further dissected into RSpW to the non-inoculated control and RGWSp to the non-inoculated control. Dunnett's analysis was carried out as described above for both RSpW and RGWSp; all lines fell into the two groups identified for AUDPC and REW (Fig. 2c, d, respectively). Correlations between the three measurements of yield (REW, RSpW, RGWSp) in 2002 were all highly significant (Table 2).

The DON content in the grain was measured for a subset of lines from two blocks of the spray-inoculated trial (2002). The parental lines had DON contents of 84 mg kg^{-1} and 190 mg kg^{-1} for HsTm4A and 'Hobbit Sib', respectively. A two-sample *t*-test conducted for DON content with the HsTm4ADH lines in the two groups as assigned by AUDPC showed the groups to differ significantly ($P < 0.001$). The average DON content of the grain was 103.1 mg kg^{-1} and 261.76 mg kg^{-1} for the resistant and susceptible groups, respectively. The mean DON levels taken for each genotype over two blocks were highly positively correlated with AUDPC, and highly negatively correlated with RSpW, $r = 0.904$ and -0.935, 1 *df*, $P < 0.001$, respectively (Table 2).

Point inoculation

A two-sample *t*-test showed no significant difference between the parental lines for the number of spikelets infected at 7, 14 or 21 dpi ($P > 0.05$). The frequency distribution of visual disease symptoms of the DH lines followed a normal distribution centred about the two parental lines, indicating the absence of type II resistance (Fig. 3).

Genotyping

Only three microsatellite markers (*Xgwm165*, *Xgwm601* and *Xgwm610*) were polymorphic between 'Hobbit Sib' and HsTm4A. All the HsTm4A DH lines were genotyped using these microsatellites. The location and order of the microsatellites were confirmed using the ITMI 'Opata85' × W-7984 RI population in conjunction with the RFLP mapping data set (Fig. 4). Due to the paucity of suitable microsatellite markers, SSAP markers were also used to characterize the 46 HsTm4ADH lines to produce a map of this population (Fig. 4.) Using marker/phenotype association, it was possible to determine the location of the FHB resistance on chromosome 4A of *T. macha*. Recombination between FHB resistance and microsatellite markers *Xgwm601* and *Xgwm610* was observed in the HsTm4ADH population with some resistant lines carrying the 'Hobbit Sib' alleles for these markers. However, all the HsTm4ADH lines carrying the HsTm4A allele for *Xgwm165* were in the 'resistant' grouping for AUDPC, REW, RSpW and RGWSp, and

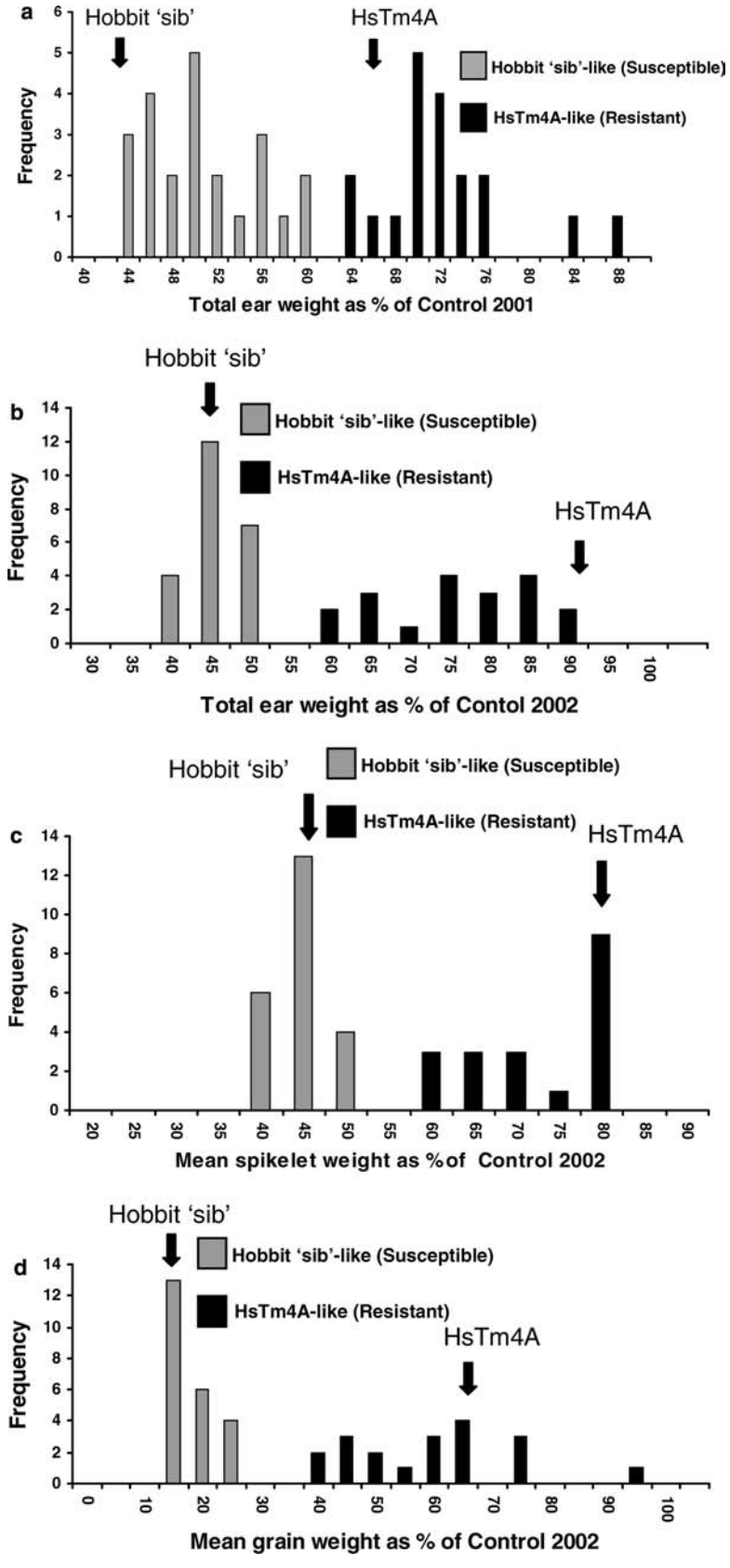

Fig. 2 Frequency distribution of 43 spray-inoculated DH lines for total ear weight as a percentage of non-inoculated controls in 2001 (**a**) and 2002 (**b**). Groups were determined by Dunnet's one-way analysis of variance as 'Hobbit Sib'-like (*susceptible*) or HsTm4A-like (*resistant*). Values for parental genotypes are indicated. **c** Frequency distribution of 43 spray-inoculated DH inbred lines for mean spikelet weight as a percentage of non-inoculated controls in 2002. Groups were determined by Dunnet's one-way analysis of variance as 'Hobbit Sib'-like (*susceptible*) or HsTm4A-like (*resistant*). Values for parental genotypes are indicated. **d** Frequency distribution of 43 spray-inoculated DH inbred lines for mean grain weight per spikelet as a percentage of non-inoculated controls in 2002. Groups were determined by Dunnet's one-way analysis of variance as 'Hobbit Sib'-like (*susceptible*) or HsTm4A-like (*resistant*). Values for parental genotypes are indicated

all the susceptible lines carried the 'Hobbit Sib' allele for this microsatellite. Thus, by comparison to the ITMI map the FHB resistance on chromosome 4A of *T. macha* was deduced to reside distal to microsatellite marker *Xgwm601*, co-segregating with *Xgwm165* in the HsTm4ADH population.

Table 2 Correlation coefficients between phenotypic traits from spray inoculation experiments in 2001 and 2002

Trait	AUDPC[a] 2001	AUDPC 2002	REW[b] 2002	RSpW[a] 2002
AUDPC 2002	0.854			
REW 2001	−0.913			
REW 2002	–	−0.962		
RSpW 2002	–	−0.970	0.820	
RGWSp[b] 2002	–	−0.926	0.941	0.955
DON[c] 2002	–	0.904	–	−0.935

[a] *RSpW* Average spikelet weight of inoculated ears at harvest relative to non-inoculated controls
[b] *RGWSp* Average grain weight per spikelet of inoculated ears at harvest relative to non-inoculated controls
[c] *DON* Deoxynivalenol content from a randomly selected subset of 22 lines from two blocks

Discussion

Resistance to FHB has been shown in numerous studies to be under oligo-/polygenic control (Beurstmayr et al. 1997; Ban and Suenaga 2000), with genes on several chromosomes contributing to the resistance through both passive (Cook 1981; Mesterhazy et al. 1999) and active resistance mechanisms (Schroeder and Christiansen 1963; Miller and Arnison 1986; Mesterhazy et al. 1999). Two previous studies have shown that the resistance to FHB in *T. macha* is located on several chromosomes, but that chromosome 4A confers much of this resistance (Grausgruber et al. 1998; Mentewab et al. 2000). Generally, the incomplete nature of FHB resistance, significant environmental influences and the complex biology of the disease make it difficult to phenotype lines for resistance and hinders the subsequent development of resistant cultivars. Different research groups have identified various numbers of genes influencing resistance in the same variety. For example, Van Ginkel et al. (1996) concluded that the variety 'Frontana' has two major genes controlling the overall resistance, whereas Singh et al. (1995) identified 'Frontana' as having three major genes. Many published studies have identified QTL for FHB resistance. The most studied source 'Sumai 3' is a key example of the use of mapping populations to identify QTL for disease resistance. Various numbers of genes (QTL), with differing chromosomal locations, and different contributions to the total resistance have been proposed (Bai et al. 1999; Ban and Suenaga 2000; Anderson et al. 2001; Ruckenbauer et al. 2001; Beurstmayer et al. 2002). The lines used in the present work differ from those used in the above studies in that they are essentially homozygous for all chromosomes other than the target chromosome 4A. This eliminates any interaction of resistance genes residing elsewhere in the genome. Stack et al. (2002) used a similar approach with a substitution series of *Triticum dicoccoides* in a partially resistant durum wheat cultivar ('Langdon'), and identified chromosome *T. dicoccoides* 3A as having a significant effect on FHB resistance. Otto et al. (2002) subsequently identified a QTL for type II resistance in a 29.3-cM centromeric region, using recombinant inbred chromosome lines generated from a cross between the 'Langdon' (*T. dicoccoides* 3A) and 'Langdon'. Unlike the partially resistant 'Langdon', 'Hobbit Sib' is extremely susceptible to FHB, and this allowed a clear resolution between resistant and susceptible phenotypes among the DH recombinants used in the present study. After spray inoculation, the lines separated in a bimodal fashion into resistant and susceptible groups for AUDPC and REW, and this is likely to be due to the presence of a single gene or closely linked genes inherited from *T. macha* located on the short arm of chromosome 4A.

Following point inoculation, no significant difference in the rate of spread of the fungus was observed between the parental genotypes, and the HsTm4A DH lines gave a normal distribution for visual disease centred about the parents. A similar point inoculation experiment was performed in 2004 (results not shown), and again there was no significant difference between the resistant and susceptible groups as assigned by the spray inoculation AUDPC. Thus, the resistance conferred by *T. macha* 4A does not act predominantly through resistance to spread of the fungus within the ear (type II resistance). The resistance evident with the spray-inoculated trials is therefore resistance to initial infection, known as type I. Paillard et al. (2004) described a QTL for combined type I and type II field resistance to FHB on the long arm of

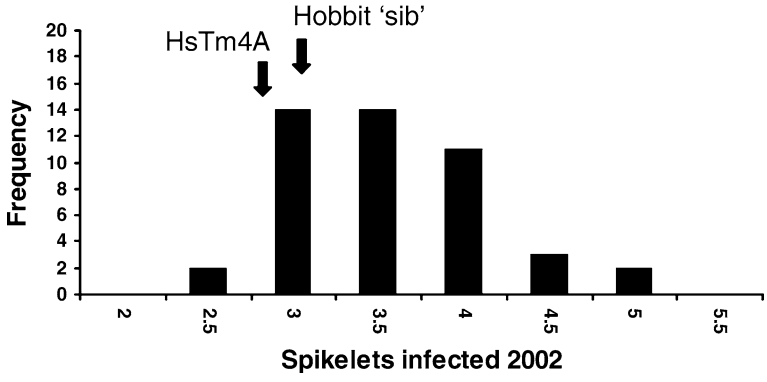

Fig. 3 Frequency distribution of 43 point-inoculated DH lines for number of spikelets infected 3 weeks post-inoculation in 2002. Values for parental genotypes are indicated

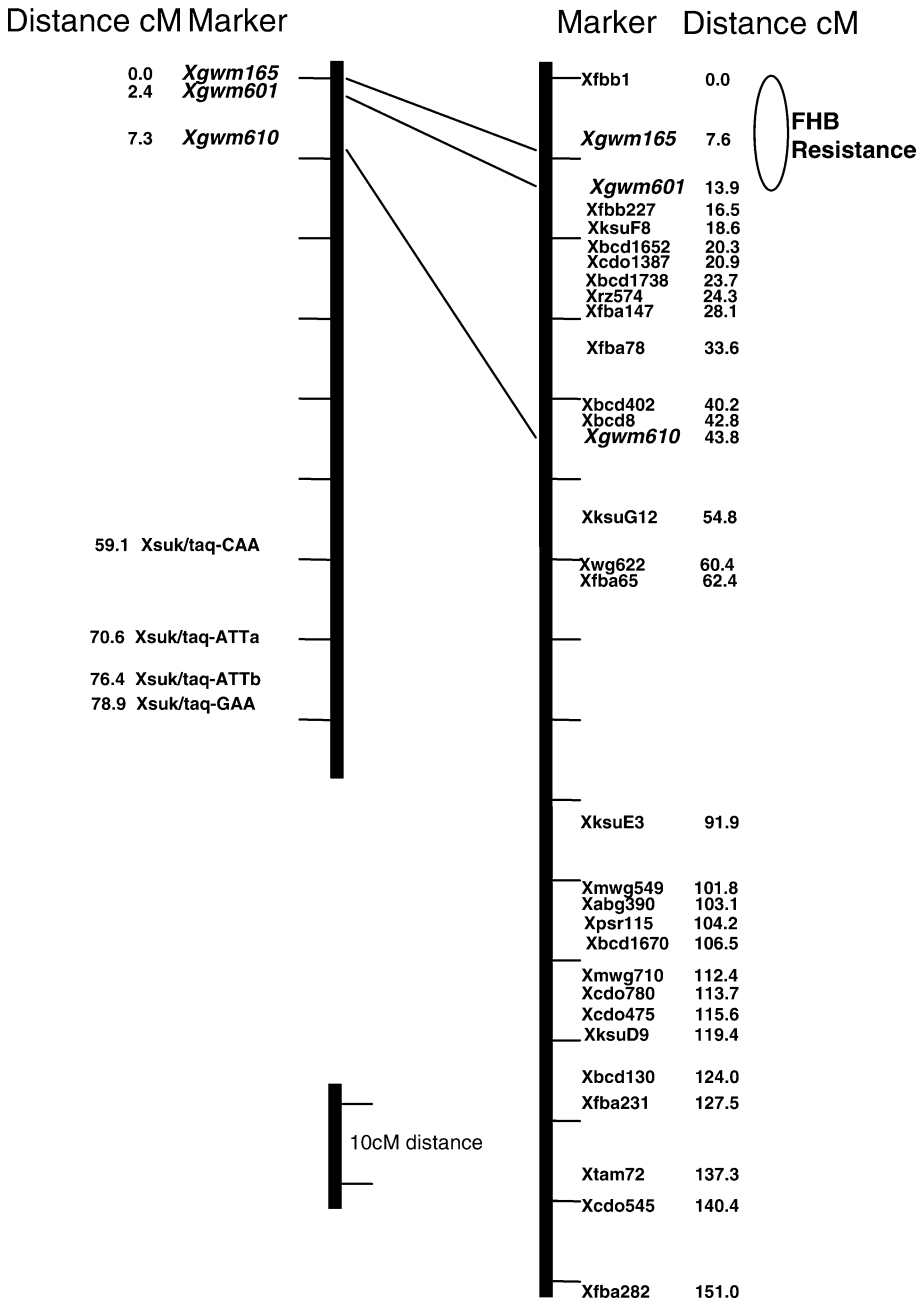

Fig. 4 Genetic maps of chromosome 4A from the 'Hobbit Sib' × HsTm4A DH population constructed using microsatellite and sequence-specific amplified polymorphism markers and *Triticum aestivum* chromosome 4A constructed using microsatellite genetic markers mapped from the International Triticeae Mapping Initiative (ITMI) 'Opata85' × W-7984 RI population in conjunction with the ITMI RFLP data set. Microsatellite markers shown in *italics* were used to genotype the 'Hobbit Sib'(*T. macha* 4A) DH lines

chromosome 4A, derived from the Swiss winter wheat variety 'Arina'. The resistance described in this paper derived from *T. macha* is putatively the first report of an FHB type I resistance locus located on the short arm of wheat chromosome 4A. We propose this locus be named *QFhs.jic-4AS*.

The mechanism underlying type I resistance is still unclear. Schroeder and Christensen, who first described the resistance in 1963, assumed it to be physiological, as they found no evidence of it being anatomical or histological in nature. Some authors have questioned whether it is a true resistance being perhaps associated with disease avoidance, (Parry et al. 1995). In the present study, many of the elements associated with disease escape, such as plant height (Jenkinson and Parry 1994) and flowering time, were removed by inoculating the ear directly at anthesis. All lines were inoculated with a high concentration of inoculum at mid-anthesis, the most susceptible developmental period for FHB infection (Strange and Smith 1971; Parry et al. 1995). The open florets allow the pathogen to enter and contact the ovary and the inner surfaces of the palea, glume and lemma, the site at which the host is first infected (Kang and Buchenauer 2000). High humidity and temperature, important for disease development, were also maintained. Most resistances reported for FHB to date are primarily of type II (Ban and Suenaga 2000; Anderson et al. 2001; Buerstmayr et al. 2002), and research has generally concentrated on this type of resistance as it has been considered both simple to target and more robust

than type I (Buerstmayr et al. 2003). The present study has demonstrated that type I resistance present on *T. macha* 4A can provide considerable protection under intense disease pressure from the initial infection by the fungus. The resistance can also be considered to be relatively stable, as it has been expressed in glasshouse, polytunnel and field environments (Grausgruber et al. 1998; Mentewab et al. 2000).

Preventing the accumulation of mycotoxins in grain is one of the most important food safety issues concerning agriculture today. The *T. macha* 4A resistance resulted in a significant reduction in the accumulation of DON in grain. The potential importance of the type I resistance conferred by the *QFhs.jic-4AS* locus to the breeding of FHB resistant wheat is clearly demonstrated by the reduction in both disease levels and DON accumulation. It has been shown by Bai et al. (2000) that there is generally an additive effect in terms of resistance when resistances are brought together from diverse sources. Combining different resistance mechanisms to FHB such as those for type II, found on chromosome 3B of 'Sumai 3' and 3A of *T. dicoccoides* (Anderson et al. 2001; Stack et al. 2002), with the stable type I resistance of *T. macha* 4A, could therefore provide an effective method for combating initial infection as well as spread of the fungus within the spike. It should also provide a means of addressing the important issue of reducing the potential for contamination of grain with mycotoxins. We are presently carrying out further backcrosses using the *T. macha* 4A recombinant DH lines into the 'Hobbit Sib' background to refine the map position of *QFhs.jic-4AS*.

Acknowledgements This work was funded partly through an EU-INCO. DC project (ERBIC18 CT98 0312). We are grateful for the technical support provided by Jane Kirby (JICgenotyping@jicgenomelab.co.uk, http://www.JICgenomelab.co.uk) with regard to genotyping the DH lines. We also thank Henry Nash and Richard Draeger for their help with the collection of phenotypic data.

References

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

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

Bai GH, Shaner G, Ohm H (2000) Inheritance of resistance to *Fusarium graminearum* in wheat. Theor Appl Genet 100:1–8

Ban T (2000) Analysis of quantitative trait loci associated with resistance to fusarium head blight caused by *Fusarium graminearum* Schwabe and of resistance mechanisms in wheat (*Triticum aestivum L.*). Breed Sci 50:131–137

Ban T, Suenaga K (2000) Genetic analysis of resistance to Fusarium head blight caused by *Fusarium graminearum* in Chinese wheat cultivar Sumai 3 and the Japanese cultivar Saikai 165. Euphytica 113:87–99

Berek L, Petri IB, Mesterhazy A, Teren J, Molnar J (2001) Effects of mycotoxins on human immune functions in vitro. Toxicol In Vitro 15:25–30

Bryan GJ, Collins AJ, Stephenson P, Orry A, Smith JB, Gale MD (1997) Isolation and characterisation of microsatellites from hexaploid bread wheat. Theor Appl Genet 94:557–563

Buerstmayr H, Lemmens M, Ruckenbauer P (1997) Chromosomal location of Fusarium head blight resistance genes in wheat. Cereal Res Commun 25:731–732

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

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor Appl Genet 107:503–508

Cook RJ (1981) *Fusarium* diseases of wheat and other small grains in North America. In: Nelson PE, Touson TA, Cook RJ (eds) *Fusarium* diseases, biology and control. Pennsylvania State University Press, University Park, pp 39–52

Dardis JV, Walsh EJ (2003) An evaluation of exotic germplasm as sources of fusarium head blight resistance in bread wheat (*Triticum aestivum* L). Cereal Res Commun 31:129–136

Desjardins AE, Proctor RH, Bai GH, McCormick SP, Shaner G, Buechley G, Hohn TM (1996) Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. Mol Plant–Microbe Interact 9:775–781

Gilbert J, Tekauz A (2000) Review: recent developments in research on fusarium head blight of wheat in Canada. Can J Plant Pathol 22:1–8

Gosman NE (2002) Analysis of the genetic basis of resistance to *Fusarium culmorum* in wheat. PhD Thesis, University of East Anglia

Grausgruber H, Lemmens M, Burstmayr H, Ruckenbauer P (1998) Chromosomal location of Fusarium head blight resistance and in vitro toxin tolerance in wheat using the 'Hobbit Sib' (*Triticum macha*) chromosome substitution lines. J Genet Breed 53:173–180

Harris LJ, Desjardins AE, Plattner RD, Nicholson P, Butler G, Young JC, Weston G, Proctor RH, Hohn TM (1999) Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. Plant Dis 83:954–960

Jenkinson P, Parry DW (1994) Splash dispersal of conidia of *Fusarium culmorum* and *Fusarium avenaceum*. Mycol Res 98:506–510

Joffe AZ (1983) Environmental-conditions conducive to *Fusarium* toxin formation causing serious outbreaks in animals and man. Vet Res Commun 7:187–193

Kalendar R, Grob T, Regina M, Suoniemi A, Schulman A (1999) IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theor Appl Genet 98:704–711

Kang ZS, Buchenauer H (2000) Cytology and ultrastructure of the infection of wheat spikes by *Fusarium culmorum*. Mycol Res 104:1083–1093

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

Laurie DA, Reymondie S (1991) High-frequencies of fertilization and haploid seedling production in crosses between commercial hexaploid wheat varieties and maize. Plant Breed 106:182–189

Liu SX, Anderson JA (2003) Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. Crop Sci 43:760–766

Mentewab A, Rezanoor HN, Gosman N, Worland AJ, Nicholson P (2000) Chromosomal location of Fusarium head blight resistance genes and analysis of the relationship between resistance to head blight and brown foot rot. Plant Breed 119:15–20

Mesterhazy A, Bartok T, Mirocha CG, Komoroczy R (1999) Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breed 118:97–110

Miller JD, Arnison PG (1986) Degradation of deoxynivalenol by suspension-cultures of the fusarium head blight resistant wheat cultivar Frontana. Can J Plant Pathol 8:147–150

Milus EA, Parsons CE (1994) Evaluation of foliar fungicides for controlling fusarium head blight of wheat. Plant Dis 78:697–699

Nicholson P, Lees AK, Maurin N, Parry DW, Rezanoor HN (1996) Development of a PCR assay to identify and quantify *Microdochium nivale* var *nivale* and *Microdochium nivale* var *majus* in wheat. Physiol Mol Plant Pathol 48:257–271

Otto CD, Kianian SF, Elias EM, Stack RW, Joppa LR (2002) Genetic dissection of a major Fusarium head blight QTL in tetraploid wheat. Plant Mol Biol 48:625–632

Paillard S, Schnurbusch, Tiwari R, Messmer M, Winzeler M, Keller B, Schachermayr (2004) QTL analysis of resistance to Fusarium head blight in Swiss winter wheat (*Triticum aestivum* L.). Theor Appl Genet 109:323–332

Parry DW, Jenkinson P, Mcleod L (1995) Fusarium ear blight (scab) in small-grain cereals—a review. Plant Pathol 44:207–238

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

Ruckenbauer P, Buerstmayr H, Lemmens M (2001) Present strategies in resistance breeding against scab (*Fusarium* spp.). Euphytica 119:121–127

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

Shen X, Zhou M, Lu W, Ohm H (2003) Detection of Fusarium head blight resistance QTL in a wheat population using bulked segregant analysis. Theor Appl Genet 106:1041–1047

Simpson DR, Weston GE, Turner JA, Jennings P, Nicholson P (2001) Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. Eur J Plant Pathol 107:421–431

Singh RP, Ma H, Rajaram S (1995) Genetic-analysis of resistance to scab in spring wheat cultivar Frontana. Plant Dis 79:238–240

Snijders CHA (1990) Genetic-variation for resistance to fusarium head blight in bread wheat. Euphytica 50:171–179

Snijders CHA, Perkowski J (1990) Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. Phytopathology 80:566–570

Stack RW, Elias EM, Fetch JM, Miller JD, Joppa LR (2002) Fusarium head blight reaction of Langdon durum—*Triticum dicoccoides* chromosome substitution lines. Crop Sci 42:637–642

Stam P, van Ooijen (1995) JoinMap version 2.0 Software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen

Strange RN, Smith H (1971) A fungal growth stimulant in anthers which predisposes wheat to attack by *Fusarium graminearum*. Physiol Plant Pathol 1:141–150

Van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorak J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995) Molecular-genetic maps for group 1 chromosomes of *Triticeae* species and their relation to chromosomes in rice and oat. Genome 38:45–58

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

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP—a new technique for DNA-fingerprinting. Nucleic Acids Res 23:4407–4414

Wan YF, Yen C, Yang JL, Liu FQ (1997) Evaluation of Roegneria for resistance to head scab caused by *Fusarium graminearum* Schwabe. Genet Resour Crop Evol 44:211–215

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

Yang ZP, Gilbert J, Somers DJ, Fedak G, Procunier JD, McKenzie IH (2003) Marker assisted selection of Fusarium head blight resistance genes in two doubled haploid populations of wheat. Mol Breed 12:309–317

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421