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A monoclonal antibody chromosome marker analysis used to locate a loose smut resistance gene in wheat chromosome 6A

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Abstract Many genes have been located in wheat chromosomes, yet little is known about the location of genes for resistance to *Ustilago tritici*, which causes loose smut. Crosses were made between the loose smut susceptible alien substitution lines Cadet 6Ag(6A) and Rescue 6Ag(6A) (lines in which *Agropyron* chromosome 6 is substituted by wheat chromosome 6A) and four cultivars resistant to *U. tritici* race T19: 'Cadet', 'Kota', 'Thatcher' and 'TD18'. The segregating progeny were tested for reaction to race T19 and for the level of binding with a monoclonal antibody specific to a chromosome 6A-coded seed protein. The antibody, which does not bind to seed protein extracts in the absence of the 6A chromosome, was used as a chromosome marker. An association was established between resistance to race T19 and the presence of chromosome 6A for each of the cultivars tested, indicating that resistance to race T19 resides in chromosome 6A. *Ustilago tritici* race T19 resistance in 'Cadet' appears to be located in the short arm of chromosome 6A, based on the evaluation of the Cadet 6A long ditelosomic stock, which was susceptible, and the Cadet 6A-short: 6-*Agropyron*-short alien translocation stock, which was resistant.

Key words *Triticum aestivum* · *Ustilago tritici* · Alien substitution · Molecular marker · Gene location

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Introduction

The genetic analysis of loose smut resistance is difficult (Ribeiro 1963), and no attempt has yet been made to locate the genes coding for resistance to this disease to chromosomes using monosomic analysis. Information regarding the chromosomal location of loose smut resistance genes would be useful for efficient breeding and for the development of molecular markers to resistance genes (Anderson et al. 1993).

Loose smut resistance is difficult to study because florets of the wheat spike must be individually inoculated and progeny testing requires extensive growth cabinet and greenhouse space (Jones and Dhitaphichit 1991). Evaluation of loose smut reaction is done on a family basis (Gaskin and Schafer 1962), requiring nearly two full generations, one for inoculation and the second for symptom expression. Because of the wide variation in loose smut reaction within a genotype, genetic analysis is complicated by overlap in the reaction of resistant and susceptible families such that delimitation of the classes is difficult (Ribeiro 1963). However, arbitrary delimitation of classes is undesirable (Knott 1987).

Dhitaphichit et al. (1989) analyzed two chromosome substitution series to locate two loose smut resistance genes to wheat chromosomes 7A and 7B. Results from analyses of varietal substitution lines, however, have not provided conclusive evidence of the location of a gene (McIntosh 1987; Koebner et al. 1988; Yen and Baenziger 1992). Such backcross lines are prone to incomplete genotype restoration. Furthermore, heterogeneity or residual heterozygosity in either or both the recurrent and donor parents used in the development of substitution lines can result in misleading conclusions.

Procedures involving segregation analysis, when confirmed cytogenetic stocks are used, not only provide stronger evidence of the chromosomal location of a gene, but also allow the analysis of traits not carried within existing cytogenetic stocks. Monosomic analysis with segregating populations is commonly used for locating genes in

chromosomes (Sears 1969). This method, however, requires large F_2 populations in order to distinguish segregation ratios.

Knox et al. (1992) presented a chromosome marker method of segregation analysis in which crossing over of a particular chromosome is avoided by the use of nullisomic cytogenetic stocks. Chromosomal segregation is followed using seed proteins as chromosome-specific molecular markers. This method is appealing because genes can be located to chromosomes using relatively small populations. A further advantage is the rapidity of the analysis using monoclonal antibodies (MAb) to seed proteins (Howes et al. 1989). MAbs are also used as chromosome markers in populations involving alien substitution lines (Howes et al. 1994). These lines could be used in place of nullisomic lines, as has been suggested by Knox et al. (1992).

The study presented here was undertaken to provide evidence of the chromosome location of a loose smut resistance gene or genes and also to validate the use of MAbs as molecular chromosome markers in conjunction with alien substitution lines. The approach used was to evaluate a variety of sources of resistance against those chromosomes for which good seed protein markers are available (rather than using a full series of 21 alien chromosome substitution lines crossed onto a single source of resistance). The group 1 and group 6 wheat chromosomes code for the seed storage proteins (Payne 1987) and offered the greatest potential for polymorphic chromosome markers. Cytogenetic stocks representing group 1 and 6 chromosomes were crossed with different sources of loose smut resistance. The sources of resistance chosen for evaluation included lines from the *U. tritici* race differential set (Nielsen 1987), other sources of resistance of historical significance in spring wheat breeding and the progenitors of cytogenetic stocks found to be resistant during the course of this study.

Materials and methods

Eight races of *U. tritici* (T2, T6, T8, T10, T15, T19, T31 and T39) were selected to maximize virulence within each race and at the same time differentiate resistance within the *U. tritici* race differentials described by Nielsen (1987). The criteria for race selection was to enhance the probability of tracking single genes for resistance (Person 1959). Cytogenetic stocks and sources of resistance were inoculated with the eight races. At least 6 months is required to evaluate a genotype for resistance. Therefore, resistance evaluation of stocks was done concurrently with crossing. Information gained from the assessment of loose smut resistance of the cytogenetic stocks and their progenitors led us to focus on resistance to race T19 in chromosome 6A.

Initially, the race T19 loose smut resistant males 'Kota' and 'TD18' were crossed onto Cadet 6Ag(6A) and Rescue 6Ag(6A), respectively. In a second series, cvs 'Cadet' and 'Thatcher' were used as race T19 resistant males crossed onto Cadet 6Ag(6A). Cadet 6Ag(6A) and Rescue 6Ag(6A) are alien chromosome substitution lines in which the 6A chromosome is substituted with the homoeologous *Agropyron elongatum* chromosome 6Ag (Whelan 1988). F_1 plants were grown from each cross, and the spikes were bagged. Only races T8, T15, T19 and T31 were pertinent for the parental combinations described.

Seed of the F_2 generation was analyzed for the presence or absence of the wheat 6A chromosome, based on the binding of MAb 230/9 (Skerritt et al. 1991). Antibody binding was based on an ELISA (Enzyme Linked Immunosorbant Assay) described by Howes et al. (1989). A portion of the brush end of each seed was removed and placed in a multiwell plate, and then extracted in 150 ul of 50% propan-2-ol overnight at 37°C. Seed pieces were squashed with a blunt rod and allowed to incubate another 30 min. Three replicates of a 1:10 dilution of each extract in 70% ethanol were bound to a plate at room temperature for 30 min, then blocked with 1% milk for 15 min. The MAb 230/9 was diluted 1:10,000 and applied to plates for 90 min; this was followed by the addition of goat-antimouse antibody conjugated with alkaline phosphatase diluted 1 in 2,000, and the entire complex was incubated for 2 h. Ethanolamine buffer (1.0 *M*, pH 9.5) containing 1 mg/ml *p*-nitrophenyl phosphate as substrate for the phosphatase was added, and the color reaction was allowed to develop. Absorbance was measured using a Titre Tek multichannel spectrophotometer at wavelength 405 nm. Incubations of the milk and antibody steps were made at 37°C. Washes between each step used 20 m*M* TRIS pH 7.4–0.15 *M* NaCl–0.05% Tween 80 (TBS -Tween). Antibodies were prepared in 0.05% milk diluted in TBS-Tween.

Up to 80 F_2 seeds from each population were analyzed with MAb 230/9, along with 'Thatcher' and 'Cadet', which were used as high-antibody-binding-controls. Ten of the highest binding seeds, expected to be disomic for the wheat 6A chromosome, and 10 of the lowest binding seeds, expected to lack 6A but be disomic for the 6Ag chromosome, were selected (Howes et al. 1994). Absorbance readings were converted to relative readings using the high controls as a basis. The population size of 10 high-binding and 10 low-binding seeds was determined based on the ability to distinguish the two most likely causes of the expected outcome of all low-binding seed (disomic 6Ag) selections being susceptible and all high-binding seed (disomic 6A) selections being resistant. These two causes are either chance segregation or a real association between loose smut resistance and the wheat chromosome.

The embryo ends of the seeds with the desired antibody reactions were pregerminated and planted. Sterile soil was used to minimize microbial infestation of the seed and prevent foreign seed contamination. The Cadet 6Ag(6A)/'Kota' population was inoculated with race T19 as a priority and, if fertile spikes remained, T31 was also inoculated to determine the association between T31 and T19 resistance. Likewise, the Rescue 6Ag(6A)/'TD18' population was inoculated with T19 first, and T15, T31 and T8 were inoculated onto later spikes. The Cadet 6Ag(6A)/'Cadet' and Cadet 6Ag(6A)/'Thatcher' populations were inoculated with race T19 only.

Plants to be inoculated were grown in a growth cabinet at 18°C, under 16 h light and 8 h dark. Spikes were inoculated using a 10 ml hypodermic syringe with a 22-gauge needle to inject a water suspension of spores (approximately 1 mg/ml) into florets at mid-anthesis. Following inoculation, the spike was bagged to minimize cross pollination and to improve infection conditions (Nielsen 1987).

A minimum family size for loose smut evaluation was calculated using loose smut values of the parental lines (Table 1). The family size needed to distinguish between p1=0.74 [the probability of occurrence of factor one, here equal to the proportion of smutted plants in Cadet 6Ag(6A)] and p2=0.26 (the probability of occurrence of factor two, here equal to the proportion of smutted plants in 'Kota') at a 99% probability is 16 (Hanson 1959) for the population Cadet 6Ag(6A) by 'Kota'. This combination of parents required the greatest family size of any of the parental combinations studied. Where possible, at least 2 spikes (often 3–4 spikes) on each F_2 plant were inoculated. Because of the statistical unreliability of data from families of fewer than 16, such data were not used for the analysis and interpretation. However, it should be noted that inclusion of data from families of fewer than 16 would not have distorted interpretation of the results.

Inoculated spikes were harvested and threshed separately at maturity. Seed from 1 spike from each family for each race was planted in the greenhouse, followed by planting of a second spike when the space became available, and so on until all spikes had been planted and evaluated. The number of loose smut infected plants and of

Table 1 Proportion of smutted to total plants (P) and total plants (N) of wheat cultivars and lines inoculated with loose smut races T19, T31, T15 and T8 and monoclonal antibody 230/9(OD) relative to 'Cadet' and the standard deviation (SD) using a sample size of 6 (– Test not done)

Race	T19		T31		T15		T8		OD	SD
	P	N	P	N	P	N	P	N		
Cadet	0.11	309	0.96	28	0.00	118	0.00	104	1.00	0.295
Cadet 6Ag(6A)	0.74	204	0.93	28	0.13	103	0.00	62	0.05	0.028
Cadet6AL t″	0.55	299	–	–	–	–	–	–	0.09	0.034
Cadet 6AgS:6AS	0.22	93	–	–	–	–	–	–	1.26	0.517
Kota	0.26	23	0.00	38	0.16	37	0.65	49	0.86	0.323
Thatcher	0.16	107	0.73	56	0.00	36	0.00	59	1.05	0.361
TD18	0.00	38	0.00	35	0.00	37	0.00	36	1.08	0.054
Rescue	0.55	107	0.82	22	0.72	39	0.17	77	0.95	0.090
Rescue 6Ag (6A)	0.39	56	0.17	18	0.64	55	0.33	48	0.10	0.014

healthy plants was counted. The proportion of smutted plants was calculated; the range in family size for each population was as follows (mean family size in parentheses): Cadet 6Ag(6A)/'Kota' – T19: 18–144 (75), – T31: 16–49 (36); Cadet 6Ag(6A)/'Thatcher' - T19: 34–106 (66); Cadet 6Ag(6A)/'Cadet' - T19: 48–142 (102); Rescue 6Ag(6A)/TD18 – T8: 18–71 (45), – T15: 19–107 (61), – T19: 19–112 (60), – T31: 21–112 (41).

To confirm the MAb 230/9 binding of the single F_2 seeds, 10 F_3 seeds from the populations Cadet 6Ag(6A)/'Kota', Cadet 6Ag(6A)/'Thatcher' and Cadet 6Ag(6A)/'Cadet' were tested against the antibody in an ELISA.

The alien translocation line Cadet 6AgS:6AS (Whelan and Lukow 1990), in which the long arm of chromosome 6A of 'Cadet' is replaced by the short arm of chromosome 6Ag from *Agropyron*, and the ditelosomic Cadet 6AL t″, which misses the short arms of the homologous 6A chromosomes, were inoculated to provide information on the arm location of the resistance gene. These inoculations were made with race T19 after the reaction to race T19 of the Cadet 6Ag(6A) and Cadet had been determined. There was no reason to inoculate the translocation and ditelosomic lines to the other races.

A *t*-test was performed on the absorbance readings of the parents (Table 1). 'Thatcher', 'Cadet', 'Kota' and Cadet 6Ag(6A), and 'TD18' and Rescue 6Ag(6A) loose smut reactions were compared using Fisher's exact two-tail test. Pairwise, 2×2 tests of independence using Fisher's exact two-tail test were made using the loose smut reaction data from 'Cadet', Cadet 6Ag(6A), Cadet 6AgS:6AS and Cadet 6AL t″. A *t*-test could not be used because values of the resistant cultivars were not normally distributed. Loose smut data from the segregating populations were arcsine transformed to obtain normality before applying a *t*-test. All tests were run using the computer procedures of the Statistical Analysis System Institute (1989).

Results

The results presented are from the populations that showed T19 resistance associated with chromosome 6A. The reactions of these populations to other races are also presented to provide a contrast between resistance associated with and resistance not associated with the wheat 6A chromosome.

The binding of MAb 230/9 to seed extracts of the two alien substitution lines Rescue 6Ag(6A) and Cadet 6Ag(6A) and Cadet 6AL t″ (Table 1) was significantly lower than that of the euploid cultivar 'Cadet' and Cadet 6AgS:6AS at $P< 0.005$ when the null hypothesis assumes no difference in mean absorbance readings. The difference in absorbance provided the polymorphism necessary to use this antibody as a chromosome marker.

'Cadet' and Cadet 6AgS:6AS had a clearly different response to *U. tritici* race T19 than Cadet 6Ag(6A) and Cadet 6AL t″ (Table 1). This demonstrates that the loose smut reaction was not independent of the chromosome condition ($P< 0.001$ for each of the tests). The reaction of most lines to each of the races of *U. tritici* was reasonably clear-cut; the reactions of the 'Rescue' lines were more variable (Table 1). For example, the proportion of infected plants was 0.17 for Rescue 6Ag(6A) inoculated with race T31, whereas this proportion in 'Rescue' was 0.82 (n=22). 'Thatcher' and 'Kota' differed significantly from Cadet 6Ag(6A), and 'TD18' from Rescue 6Ag(6A), in their reaction to *U. tritici* race T19 ($P< 0.001$).

MAb 230/9 binding to F_2 seed extracts represents the wheat chromosome dosage (Knox et al. 1992). The results of ELISAs for the four populations are shown in Fig. 1. The seed producing low-MAb-binding extracts are represented by the far left-hand tail of the distribution and lacked the wheat 6A chromosome. Embryos of these seeds were expected to be mostly disomic for the 6Ag chromosome (endosperm trisomic 6Ag). The high-MAb-binding seed extracts making up the far right-hand tail of the distribution were expected to possess embryos disomic for the 6A chromosome (endosperm trisomic 6A). The Cadet 6Ag(6A)/'Kota' population produced a trimodal distribution (Fig. 1B), as did the Rescue 6Ag(6A)/'TD18' population (Fig. 1D), whereas the Cadet 6Ag(6A)/'Cadet' (Fig. 1A) produced few low-reacting seed, and Cadet 6Ag(6A)/-'Thatcher' produced none (Fig. 1C). The seed with extremely high or extremely low MAb binding were subjected to loose smut analysis and further MAb analysis. The intermediate-binding seed were expected to be double monosomics and, with the exception of the 'Thatcher' population, were excluded from further study. A 10-seed sample from each F_2 plant (F_3 seed) was used to confirm the reaction of the single F_2 seeds. The number of double monosomics accidentally selected was 1 in 20 for the Cadet 6Ag(6A)/'Kota' population, and 5 in 20 for the Cadet 6Ag(6A)/'Cadet' population, where the 20 seeds consisted of both low and high types. However, none of the seeds in

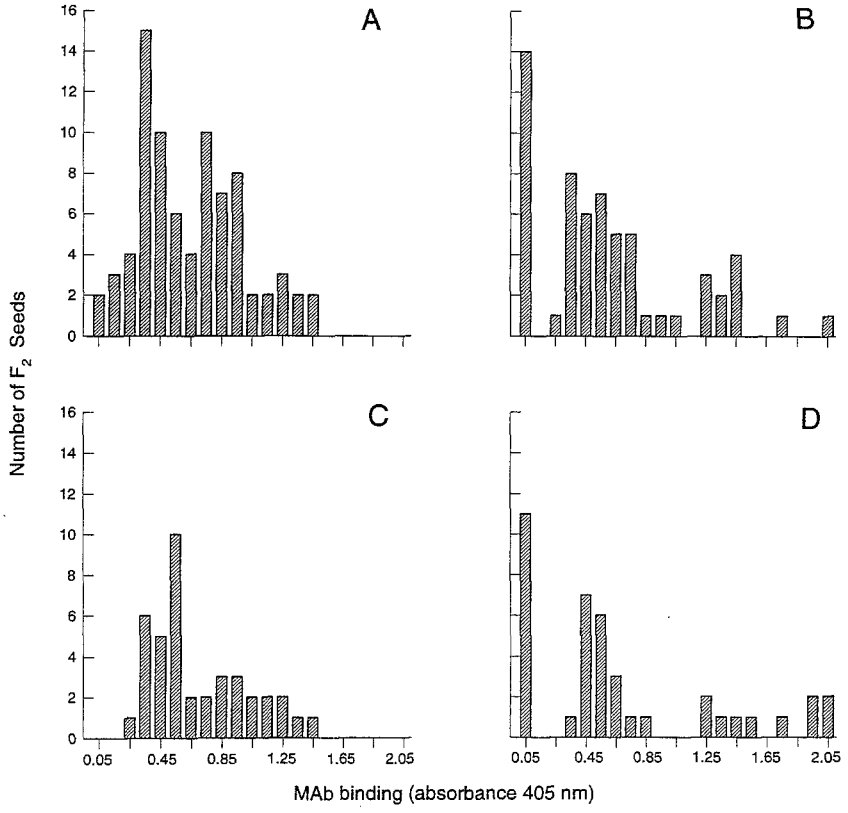

Fig. 1A–D MAb 230/9 binding measured by absorbance of an ELISA on seed extracts from F_2 populations. **A** Cadet 6Ag(6A)/'Cadet'; **B** Cadet 6Ag(6A)/'Kota', **C** Cadet 6Ag(6A)/'Thatcher', **D** Rescue 6Ag(6A)/'TD18'. Note: population sizes are not all equal

the Cadet 6Ag(6A)/'Thatcher' population were disomic for 6Ag. These results were consistent with the distributions shown in Fig. 1, which showed that very few low-binding seeds were produced by Cadet 6Ag(6A)/'Cadet' and Cadet 6Ag(6A)/'Thatcher' populations. F_3 seed of TD18 was not evaluated.

The loose smut reaction of the disomic 6A and disomic 6Ag chromosome classes are presented in Fig. 2. The result of the Cadet 6Ag(6A)/'Kota' F_2 segregation to *U. tritici* race T19 (based on the evaluation of F_3 families) is shown in Fig. 2C. Those progeny with wheat chromosome 6A had a significantly lower proportion of smut than those with 6Ag, with no overlap between the two chromosome classes (the probability of '*t*' being near infinitely small when the null hypothesis assumes no chromosome effect). The Cadet 6Ag(6A)/'Kota' population sample size was 18, divided into two groups of 9 based on chromosome classification. The probability of not obtaining the alternative phenotype in one chromosome class, based on chance segregation, was less than 0.0001. A similar response to race T19 was obtained with the Rescue 6Ag(6A)/'TD18' (Fig. 2E) and the Cadet 6Ag(6A)/'Cadet' (Fig. 2A) populations; however, the size of the alien chromosome class was only 5 for the Cadet 6Ag(6A)/'Cadet' population. The 6Ag values shown in Fig. 2B for the Cadet 6Ag(6A)/'Thatcher' population represent double monosomic plants inoculated with race T19. The distribution in response to infection with race T19 was similar for the four populations (Fig. 2). The loose smut mean of the susceptible (6Ag chromosome) families of each population was similar to that of the susceptible parent of the population. Likewise, the mean of resistant families (6A chromosome) was similar to that of the resistant parent.

'Kota' was resistant but Cadet 6Ag(6A) was susceptible to race T31. In contrast to the results obtained from inoculation with T19, when the same plants from the Cadet 6Ag(6A)/'Kota' population were inoculated with race T31 (Fig. 2D) no association between chromosome 6A and resistance could be ascertained (t-test probability is 0.65). A further illustration of the lack of association was demonstrated by the progeny of the Rescue 6Ag(6A)/'TD18' population when tested to race T15 (Fig. 2G; *t*-test probability of 0.80). The association of resistance to races T31 and T8 with the 6A chromosome was not as clear cut in the Rescue 6Ag(6A)/'TD18' population (Fig. 2F, H), although this chromosome did appear to have an influence on resistance to these races. When only the more resistant progeny, those with up to a 0.45 incidence of smut, were considered, those with the 6A chromosome seemed to be more resistant than those with the 6Ag chromosome. When a *t*-test was calculated, a significant difference between the 6A and 6Ag groups was detected at the 5% probability level.

Lines containing alien chromosomes had a lower fertility and viability than euploid plants. This was demonstrated by the number of F_3 seed and plants in each chromosome group. Also, most families that were excluded from analysis because of having too small a sample size possessed the 6Ag chromosome. As an example, essentially all of the seed of the Rescue 6Ag(6A)/'TD18' pop-

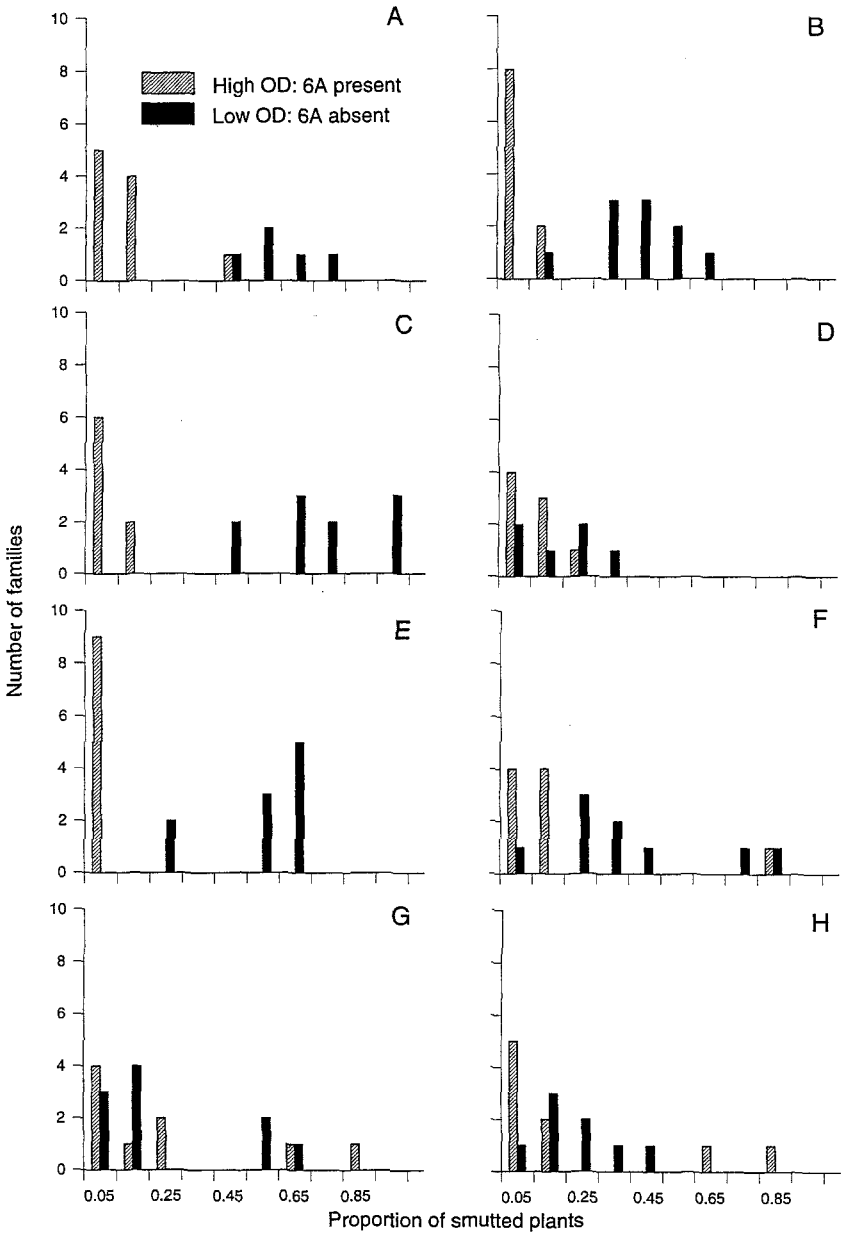

Fig. 2A–H Distribution of loose smut proportions of F_3 families, classified by chromosome on the basis of antibody readings: **A** Cadet 6Ag(6A)/'Cadet' to race T19, **B** Cadet 6Ag(6A)/'Thatcher' to race T19, **C** Cadet 6Ag(6A)/'Kota' to race T19, **D** Cadet 6Ag(6A)/'Kota' to race T31; **E** Rescue 6Ag(6A)/'TD18' to race T19, **F** Rescue 6Ag(6A)/'TD18' to race T31, **G** Rescue 6Ag(6A)/'TD18' to race T15, **H** Rescue 6Ag(6A)/TD18 to race T8. Note: population and treatment sizes are not all equal and OD is absorbance

ulation was inoculated. From the 2,982 inoculated seeds derived from plants classified as having the wheat 6A chromosome, 2,304 plants, representing the first 83 spikes inoculated, survived to be rated. Of the 2,617 inoculated seeds from plants classified as having the 6Ag chromosome, only 1,457 plants, representing seed from the first 88 spikes, survived to be rated.

Discussion

The low level of *U. tritici* race T19 infection of 'Cadet' compared to the high level of infection of Cadet 6Ag(6A) indicated that the location of resistance to race T19 is in chromosome 6A. Analysis of the Cadet 6Ag(6A)/'Cadet' population confirmed the chromosomal location of the race T19 resistance gene in the 'Cadet' 6A chromosome and demonstrated the value of the MAb-chromosome-marker/alien-substitution-line method for locating genes in chromosomes. Like monosomic analysis this method can be used to determine the chromosomal location of genes in cultivars for which cytogenetic stocks do not exist. The method was used to show that T19 resistance in 'TD18', 'Kota' and 'Thatcher' is also associated with the 6A chromosome. No explanation can be offered for the Cadet 6Ag(6A)/'Thatcher' population not generating 6Ag disomics, except perhaps that there may be poor maintenance of the alien chromosome in the disomic form in certain crosses. This may be a potential drawback to the use of alien substitution lines. However, all of the lines classed as disomic for the wheat chromosome 6A were resistant, and all of the lines double monosomic for the 6A and 6Ag chromosomes were susceptible, indicating that the suscep-

tibility of the double monosomics to race T19 is caused by a hemizygous ineffective or recessive gene. If this is the case, the evidence is strong that T19 resistance in 'Thatcher' resides in the wheat 6A chromosome. The common chromosome location of resistance to race T19 in these four cultivars is evidence that they possess the same gene, but further study is required to verify this.

The chromosome marker method appears to detect small differences in chromosome influences on a trait, as is seen in the testing of the Rescue 6Ag(6A)/'TD18' population with races T8 and T31. The use of more than one race on segregating progeny can also indicate the presence of more than one resistance gene. This was demonstrated by the Cadet 6Ag(6A)/'Kota' population inoculated to race T19 and T31 and the Rescue 6Ag(6A)/'TD18' population inoculated to race T19 and T15. The resistance to T31 in 'Kota' and to T15 in TD18 was not associated with chromosome 6A. The low loose smut reaction of Cadet 6AgS:6AS and the high reaction of Cadet 6AL t″ indicated that the T19 resistance gene in 'Cadet' is located in the short arm of chromosome 6A.

Variation in loose smut reaction exists among progeny within a chromosome class. For example, 1 segregate from the Cadet 6Ag(6A)/'Cadet' population, classified as having the 6A chromosome, was more susceptible to race T19 than its other 6A siblings (Fig. 2A). Variation, however, is also seen in parental lines. This is demonstrated by the response of 'Rescue' and Rescue 6Ag(6A) to T31 or the 0.26 incidence of loose smut in 'Kota' inoculated with race T19. This compares to a less than 0.10 incidence in the 'Kota' derived differential 'TD4' (Nielsen 1987). Variations of this type are not unusual for loose smut (Ribeiro 1963) and can be attributed to random variation, misinoculation or the segregation of other genes for partial resistance. The effect of these drawbacks can be minimized by using a large family size. In a population of families segregating for a chromosome marker and resistance, even with a very small population size, if the resistance gene were not on 6A but was segregating at random, the combined probability is remote for all lines classed as not having the 6A chromosome to segregate as susceptible and all lines classed as having the 6A chromosome to segregate as resistant. A small population used in the genetic analysis of loose smut resistance frees resources to increase family size.

Race differential lines have more value as single gene lines (Fleischmann and Baker 1971, Person 1959). 'Kota', 'Thatcher' and 'TD18' have been used as differentials (Hanna 1937; Nielsen 1987), and 'Thatcher' is a parent of 'Cadet' (Zeven and Zeven-Hissink 1976). The results of this study indicate the presence of more than one resistance gene in 'Cadet', 'Kota' and 'TD18'. Few races are virulent on 'TD18', giving further support for the possibility of at least two resistance genes (Nielsen 1987, Person 1959). Virulence studies involving 'TD4' ('Kota' derivative) and 'TD12' ('Thatcher' derivative) have also indicated that they could have more than one resistance gene (Nielsen 1977, 1982). The ease of locating loose smut resistance genes to specific chromosomes will help in identifying these genes and in developing single gene race differential lines.

The MAb 230/9 is particularly useful as a 6A chromosome marker because of its specificity and high level of binding. This antibody binds to gliadins produced at the 6A locus (Skerritt et al. 1991; Howes et al. 1994). However, MAb 230/9 does not bind to seed extracts having *Agropyron* chromosome 6 in place of chromosome 6A, as was demonstrated by this study and by Howes et al. (1994). The low antibody-binding of the Cadet 6AL t″ is consistent with the fact that the gliadin locus is located on the short arm of chromosome 6A (Garcia-Olmedo et al. 1982). To rule out random segregation with a reasonable probability, the minimum sample size for each chromosome class can be smaller than the 10 used in this study. The larger sample size is desirable, however, for reasons such as misclassification of seed, death of seedlings, or poor vigor or fertility of plants. A number of factors can cause the seed to be misclassified. These include modifier and dosage effects of genes in the endosperm (Knox et al. 1992), the environmental conditions to which the developing seed is exposed (Shewry and Miflin 1985), the size of the seed piece used for extraction, and the precision with which samples are diluted and handled.

The utility of the chromosome marker method depends on the ability to evaluate the chromosome constitution of a large initial F_2 population. This requirement is easily satisfied when a MAb to a seed protein is used as a chromosome marker. The population size required to make a decision on the chromosome location of a gene is smaller with the chromosome marker method than with monosomic analysis. Furthermore, the arbitrary division of families into resistant and susceptible classes for the determination of the segregation ratio is avoided. For these reasons, the MAb-chromosome-marker/alien-chromosome procedure is more efficient than monosomic analysis.

The MAb 230/9 is a good indicator of chromosome constitution based on the relationship achieved with loose smut reaction in the Cadet 6Ag(6A)/'Cadet' population. The method of segregation analysis used in this study can determine the chromosomal location of loose smut resistance genes as demonstrated by the localization of a T19 resistance gene to chromosome 6A in cvs 'Cadet', 'Kota', 'Thatcher' and 'TD18'. On the basis of indications from this study, other chromosome marker/alien chromosome substitution lines will be valuable tools for locating genes in chromosomes.

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