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RFLP markers linked to two Hessian fly-resistance genes in wheat (*Triticum aestivum* L.) from *Triticum tauschii* (coss.) Schmal.*

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Summary. Restriction fragment length polymorphism (RFLP) markers linked to genes controlling Hessian fly resistance from *Triticum tauschii* (Coss.) Schmal. were identified for two wheat (*Triticum aestivum* L.) germ plasm lines KS89WGRC3 (C3) and KS89WGRC6 (C6). Forty-six clones with loci on chromosomes of homoeologous group 3 and 28 clones on those of group 6 were surveyed for polymorphisms. Eleven and 12 clones detected *T. tauschii* loci in the two lines, respectively. Analysis of F_2 progenies indicated that the Hessian fly resistance gene *H23* identified in C3 is linked to *XksuH4* (6.9 cM) and *XksuG48(A)* (15.6 cM), located on 6D. The resistance gene *H24* in C6 is linked to *XcnlBCD451* (5.9 cM), *XcnlCDO482* (5.9 cM) and *XksuG48(B)* (12.9 cM), located on 3DL.

Key words: Wheat (*Triticum aestivum*) – Hessian fly – Resistance genes – RFLP markers

Introduction

Hessian fly (*Mayetiola destructor* Say) is one of the most destructive pests of wheat and has spread to almost all principal wheat-growing areas (Hatchett et al. 1987). This pest is most efficiently controlled through the use of resistant cultivars and by planting late enough to avoid infestation. More than 20 different Hessian fly resistance genes have been identified, and some have been employed in wheat breeding programs. All genes except *h4* are dominant or partially dominant. There is a gene-for-gene relationship between resistance genes in wheat and avirulence of the Hessian fly biotypes, that determines the evolution of the virulence of Hessian fly (reviewed in Gallun and Khush 1980). Kudagamage et al. (1990) reported that 12 biotypes of Hessian fly have been found in nature. The continuous evolution of virulent biotypes necessitates the identification of new resistance genes with diverse origins for wheat breeding.

Several Hessian fly resistance genes from *Triticum tauschii* (Coss.) Schmal. have been introgressed into wheat (Martin et al. 1982; Gill et al. 1986a, 1991a, b). One of these genes, *H13*, has been mapped on 6Dq (long arm) 35.0 ± 8.0 recombination units from the centromere (Gill et al. 1987). The Hessian fly resistance genes *H23* and *H24* in KS89WGRC3 (C3) and KS89WGRC6 (C6) used in this study were previously located by monosomic analysis to be on 6D and 3D, respectively (Wilson et al. 1989).

Since Botstein et al. (1980) proposed the construction of genetic linkage maps using restriction fragment length polymorphisms (RFLPs), this technology has been extensively used in genetics and molecular biology research. Because of their advantages over other genetic markers (Tanksley et al. 1989), RFLPs are well suited to be used as linked markers of disease and insect resistance genes and traits that are difficult or time-consuming to select or identify by conventional breeding methods. RFLP markers can efficiently locate alien fragments because of the high frequency of variation in the restricted DNA fragment length. Moreover, RFLP markers have been proposed to be used as the starting point for chromosome walking to clone closely linked genes in plants (Young 1990).

Although a complete RFLP linkage map of wheat is not available, a large number of RFLP markers from wheat genomic DNA, barley cDNA and oat cDNA libraries have been assigned to chromosome

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arms of wheat using nullitetrasomic and ditelosomic genetic stocks (Anderson et al. 1992). In addition, a genetic linkage map of *T. tauschii* has been published (Gill et al. 1991c). In this paper, we report the identification of RFLP markers linked to two Hessian fly resistance genes, *H23* and *H24*, transferred from two different *T. tauschii* accessions.

Materials and methods

Plant materials

Hessian fly resistance genes *H23* and *H24* in wheat germ plasms C3 and C6 were investigated in this study. C3 is a bulk F_6 progeny derived from the cross TA1642/2* 'Wichita' and C6 is a bulk F_6 progeny from cross TA2452/TA1645//2* 'Wichita'/3/ 'Newton' (Gill et al. 1991a). 'Wichita' and 'Newton' are two Hessian fly-susceptible cultivars. TA1642, TA1645 and TA2452 are three Hessian fly-resistant accessions of *T. tauschii*. All of the parents of the two germ plasm lines were used in the search for linked markers. F_2 populations were generated from crosses of 'Wichita' M-1D (monosomic 1D)/C3 and 'Wichita' M-2D (monosomic 2D)/C6. The evaluation of the resistance to biotype D of Hessian fly (*Mayetiola desturctor* Say) in F_2 populations was conducted as described by Gill et al. (1986b).

Clones

Twenty-four barley cDNA clones (BCD), 26 oat cDNA clones (CDO) and nine wheat genomic clones (WG) that have been assigned to chromosomes or chromosomal arms of homoeologous group 3 and 6 by Anderson et al. (1992) and 13 *T. tauschii* genomic clones (DG) mapped on 3D and 6D (Gill et al. 1991c) were employed in this research. All of the selected clones were applied to the screening of both accessions. The construction of the libraries and amplification of the clones were as previously described (Gill et al. 1991c; Heun et al. 1991).

DNA extraction and Southern blots

DNA from frozen leaf tissues of the parents and F_2 populations were extracted and digested as described by Heun et al. (1991). Six restriction enzymes, *Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, *Sca*I and *Xmn*I were used in the digestion of DNA for making filters to survey the clones. The first five enzymes were used in making filters for mapping polymorphic clones. Ten µg *T. tauschii* DNA and 20 µg wheat DNA were digested for each lane of the gel. The digested DNA was separated on 0.9% agarose gels and was Southern blotted onto Hybond-N^+ (Amersham) filters with 0.4 *N* NaOH transfer buffer for 6–12 h. The filters with DNA were then treated in 2 × SSC (0.3 *M* NaCl, 0.05 *M* sodium citrate) for 10 min.

Hybridization and autoradiography

Prehybridization of the filters was conducted at 65 °C in hybridization buffer (0.75 *M* NaCl, 0.125 *M* sodium citrate, 0.6% SDS, 0.05 *M* Na_2HPO_4, 5 × Denhardt's solution, 1.25 *M* EDTA and 5% dextran sulfate) for 3–6 h. PCR-amplified clones were labelled following the methods of Feinberg and Vogelstein (1983). After denaturation at 100 °C for 10 min or by treatment with 0.4 *N* NaOH solution for 10 min the labelled clones were hybridized with filters for 18–24 h.

Labelled filters were washed at 65 °C in 2 × SSC, 1 × SSC and 0.5 × SSC washing solution plus 0.05% SDS each for 15–20 min and then exposed to X-ray film for 3–7 days.

Linkage analysis

The resistance data and the RFLP data from the F_2 populations were analyzed using Mapmaker (Lander et al. 1987) to determine the linkage relationships between markers and the resistance gene. The Kosambi function (Kosambi 1944) was selected to calculate the recombination value (cM).

Results and discussion

Screening of potentially linked markers

Because cytological analysis has demonstrated that the resistance genes from C3 and C6 are on chromosome 6D and 3D, respectively (Wilson et al. 1989), clones that have been assigned to chromosomes or arms of homoeologous group 3 or 6, or mapped on 3D or 6D of *T. tauschii* (DG clones) were selected for the survey. A total of 46 clones on homoeologous group 3 and 28 on group 6 were surveyed. Two clones were counted twice because they have loci on chromosomes of both homoeologous groups. Among the 46 clones on group 3, 30 are located on 3L, 8 on 3S and the arm locations of the others are unknown. Among the 28 clones on group 6, 15 are located on 6L, 5 on 6S and 8 have unknown arm locations.

Thirty-two clones detected polymorphisms between *T. tauschii* and the hexaploid parental lines;

Table 1. Clone names and location of markers for polymorphic *T. tauschii* fragments in C3 and C6

Clones	Location	Germ plasms	
		C3	C6
CDO394	3L	+	+
CDO482	3L		+[a]
CDO718	3L	+	
CDO1396	3L	+	+[a]
BCD115	3L	+	+
BCD451	3L	+	+[a]
WG117	3L	+	+
DG G36	3D	+	+[b]
DG G48	3D/6D	+	+[a]
BCD1278	3S	+	
CDO1091	6L	+	
DG H4	6D	+	+[a]
DG G8	6D		+
BCD21	6S		+[b]
BCD342	6S		+

Under germ plasm C6, those polymorphic bands which are not specifically indicated are monomorphic between TA1645 and TA2452

+ indicates the presence of a *T. tauschii* fragment in that germ plasm line

[a] Indicates this polymorphic band from TA1645

[b] Indicates this polymorphic band from TA2452

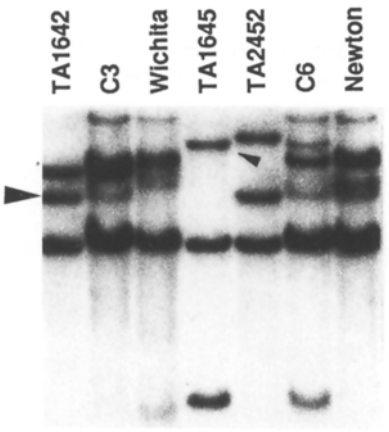

Fig. 1. DNA from five parents and two resistant lines was digested with *Hin*dIII and labelled by BCD451. The *arrows* indicate the introgressed *T. tauschii* alleles from TA1642 and TA1645 in C3 and C6, respectively. DNA hybridization was a described in Materials and methods

however, only 15 of them were identified in the two derived resistance lines (Table 1). These clones defined the *T. tauschii* fragments introduced into the chromosomes of homoeologous groups 3 and 6 of wheat and were potentially linked markers, assuming that the fragments with resistance genes are polymorphic relative to wheat loci. As shown in Table 1, some of the *T. tauschii* loci in C6 are from TA1645, and some are from TA2452. Figure 1 illustrates the detection of *T. tauschii* fragments by DNA clones. In this figure, BCD451 identified *T. tauschii* chromosomal fragments introduced into both resistance lines.

When the enzymes which produced polymorphisms for these clones were the same as those used to assign RFLP/loci to specific chromosomes in 'Chinese Spring' aneuploid stocks, the molecular size of the polymorphic loci in survey films and chromosome assignment films were compared to determine their location. The loci detected by CDO 482, BCD115 and BCD451 are on 3DL, and those by BCD21 and BCD342 are on 6DS.

Identification and mapping of linked markers

F_2 populations were produced by crossing C3 with monosomic 1D line of 'Wichita' and C6 with monosomic 2D line of 'Wichita'. Twenty-nine resistant and 11 susceptible F_2 plants were obtained from the cross of 'Wichita' M-1D with C3; 31 resistant and 6 susceptible F_2 plants were obtained from the cross of 'Wichita' M-2D with C6. χ^2 tests demonstrated that the segregation of the resistance to biotype D of Hessian fly fitted the segregation ratio of 3:1 for a single dominant gene in both populations.

Loci identified by all clones except BCD115 and 1 locus identified by DG G48 segregated 1:2:1 or 3:1 where the heterozygote could not be distinguished from one parent. Both the segregation of locus *XcnlBCD115* in the population involving C6 and the segregation of the *XksuG48(A)* locus in population involving C3 deviated significantly from the 1:2:1 segregation ratio.

Most of the clones showed no detectable linkage to the resistance genes, indicating that some *T. tauschii* chromosome fragments retained in the germ plasm lines are not linked to the resistance genes. This result was expected because the germ plasm lines were derived from one backcross to cultivated wheat for C3 and two backcrosses for C6.

In the F_2 population of 'Wichita' M-1D/C3, 11 clones located *T. tauschii* fragments; however, only *XksuH4*, on 6D, and 1 *XksuG48* locus showed co-segregation with the Hessian fly resistance. *XksuH4* is closely linked to the *H23* gene with a distance of 6.9 cM (Fig. 2a), indicating that the Hessian fly resistance gene in C3 is on 6D, consistent with the monosomic analysis of Wilson et al. (1989). The *H23* gene is linked to another Hessian fly resistance gene *H13*, also from *T. tauschii*, with a distance of about 25 cM (Gill et al. 1991a). The *XksuG48* locus is called *XksuG48(A)-6D* by referring to the map of Gill et al. (1991c). Compared with the RFLP map of *T. tauschii*, the distance (8.7 cM) between *XksuH4* and *XksuG48-(A)* in our experiment was much smaller. Presumably, crossing-over between *T. tauschii* and *T. aestivum* chromosomes was reduced. In this population, clone DG G48 hybridized to two loci. Another locus detected by DG G48 was 14.7 cM from loci *XcnlCDO394* and *XcnlBCD451*, which are completely linked. Because *XcnlBCD451* is on 3DL, this locus of *XksuG48* is inferred to be *XksuG48(B)-3D* according to the map of Gill et al. (1991c).

In the F_2 population of 'Wichita' M-2D/C6, there were 12 polymorphic markers distributed on 3D and 6D. Linkage analysis indicated that the loci identified by CDO482, BCD451 and 1 *XksuG48* locus were linked to *H24*. This *XksuG48* locus is *XksuG48(B)-3D* in accordance with its close linkage to *XcnlBCD451*, as indicated above. Figure 2b shows the linkage distances and best order between the Hessian fly resistance gene and the markers. There is no recombination between *XcnlCDO482* and *XcnlBCD451*, and these two markers are 5.9 cM from the Hessian fly gene. The close linkage to *XcnlBCD451* and *XcnlCDO482* of this gene suggests it is on 3DL. In the population of 'Wichita' M-1D/C3 *XcnlCDO451* was linked to *XcnlCDO394*; however, clone CDO394 was lost, so it could not be mapped in the C6 population.

Both CDO482 and BCD451 detected polymorphisms when *Hin*dIII and *Dra*I were used in the DNA digestion, and they showed complete linkage to each other. However, the difference in molecular weights of the polymorphic bands detected by the two clones under the same enzyme digestion indicated that they are actually located at different sites. Failure in

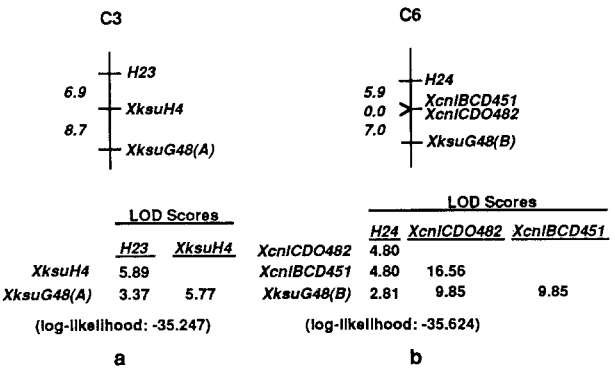

Fig. 2a, b. Linked RFLP markers for Hessian fly resistance genes *H23* in C3 and *H24* in C6. **a** Map of a fragment of chromosome 6D from C3. **b** Map of a fragment of chromosome 3DL from C6. At the *bottom* of the figure is a list of the LOD scores from grouping in two points

identifying recombinants between these two markers could be due to the reduced crossing-over within the alien *T. tauschii* chromosome fragment and/or the small population size. It has been reported that recombination between the introduced alien chromosome fragment and the corresponding host chromosome tends to be suppressed (Rick 1969; Chao et al. 1989; Ritter et al. 1991). The discrepancy between the distance of *XksuH4* and *XksuG48(A)* obtained in this study and that obtained by Gill et al. (1991c) from an F_2 population of *T. tauschii* could at least partially be attributed to suppression in our population.

C6 had two *T. tauschii* parents, TA1645 and TA2452. Among the 12 clones that located polymorphic loci in KS89WGRC6, 7 distinguished TA1645 from TA2452 (Table 1). The loci detected by the 3 linked markers in C6 all derived from TA1645, not from TA2452, indicating that the resistance gene came from TA1645, which is consistent with the result of Gill et al. (1991a). The result suggests that polymorphisms between TA1645 and TA2452 produced by the 3 markers is likely due to the segment with the resistance gene.

In both germ plasm lines linked markers were obtained for only one side of the locus. Although 46 and 28 clones on both chromosomes, including short and long arms were used, respectively, we were not able to find additional linked markers. In the D-genome map created by Gill et al. (1991c), there is also a lack of RFLP markers near *XksuG48(B)-3D*, *XksuH4* and *XksuG48(A)-6D*.

This information on linkage between RFLP markers and Hessian fly resistance genes can facilitate the screening of the resistance in breeding populations. Although combining different genes in one genotype is desirable, it is often difficult and ineffective to identify them simultaneously by traditional methods. Moreover, the expression of Hessian fly resistance genes is influenced by environmental variables such as temperature (Obanni et al. 1989). However, RFLP markers linked to the resistance genes do not have these disadvantages in selection. We can simultaneously screen multiple markers without the limitation of environment and season. Because of the close linkage of *XksuH4* with *H23*, and *XcnlCDO482* and *XcnlBCD451* with *H24*, both Hessian fly resistance genes can be tracked with marker-based selection, thus facilitating their simultaneous deployment in superior wheat varieties.

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