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Chromosomal mapping of Hessian fly-resistance gene *H13* in the D genome of wheat

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ABSTRACT: Monosomic analysis was used to locate a single, dominant, Hessian fly-resistance gene (*H13*) present in the D genome of common wheat germplasm KS81H1640HF derived from *Aegilops squarrosa* L. The seven D-genome monosomics (1D-7D) of the cultivar Wichita were crossed with KS81H1640HF and 41-chromosome F_1 plants were allowed to self. Among the seven D-genome monosomic F_2 families, six gave 3:1 segregation for the *H13* gene in reaction to Hessian fly biotype D. Critical monosomic 6D F_2 plants deviated significantly from 3:1 ratio and indicated that the *H13* gene is located on chromosome 6D. The F_3 families derived from resistant F_2 plants were again tested for *H13* gene segregation. Six monosomic F_3 families gave resistant to segregating reaction in a ratio of 1:2 and only monosomic 6D F_3 families deviated significantly from the 1:2 ratio. N-banding analysis indicated that the few segregating monosomic 6D F_3 families encountered arose from cytological abnormalities in a small fraction of the resistant F_2 plants. Telocentric analysis was used to map the *H13* gene on 6Dq (long arm) 35.0 ± 8.0 recombination units from the centromere.

COMMON WHEAT, *Triticum aestivum* L. em Thell., is an allohexaploid species (AABBDD) that originated from hybridization of a tetraploid wheat, *T. turgidum* L. (AABB), with a wild diploid species, *Aegilops squarrosa* L. (*T. tauschii*) (DD)[14]. Since its origin (ca. 8000 years), common wheat has been, to a large extent, reproductively isolated from its wild tetraploid and diploid progenitor species. As a result, most of the cultivated wheats worldwide have a relatively narrow genetic base. Because wheat is grown on more land area than any other crop, its genetic vulnerability to pests and other environmental hazards may pose a serious threat to future production. Thus, the present study is a part of long-term research aimed at broadening the genetic base of common wheat by the identification, description, and introgression of potentially useful genes among the wild relatives of wheat[7–9,11].

Among insects, the Hessian fly (*Mayetiola destructor* Say) is the most serious pest of wheat. In the United States, Hessian fly-resistant wheat cultivars have provided the most effective control. However, eight biotypes of Hessian fly have been identified in the field that attack wheats having specific genes for resistance[4,18]. Thirteen genes that condition resistance (larval antibiosis) to specific biotypes have been reported. Genes *H1*, *H2*, *H3*, *h4*, *H5*, *H7*, *H8*, and *H12* were identified in common wheat and genes *H6*, *H9*, *H10*, and *H11* were transferred from *T. turgidum* L. var. *durum* to common wheat[6,15,19–21]. Gene *H13*, resistant to the known Hessian fly biotypes, was identified in a synthetic hexaploid wheat and was shown to be derived from *A. squarrosa*[10]. More recently, we have reported several unidentified resistance genes in additional *A. squarrosa* accessions[12].

Cytogenetic and genetic studies have indicated that genes *H3*, *H6*, *H9*, and *H10* form one linkage block (ca. 36 cM long) on chromosome 5A and that genes *H5* and *H11* form another linkage block (ca. 4 cM long) on chromosome 1A[5,16,17,19,20]. The genome sources and chromosomal location of the other genes have not been determined. In this communication, we report the chromosomal location and telocentric mapping of the *H13* gene on chromosome 6D of wheat.

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Materials and Methods

The single, dominant gene, *H13*, present in wheat germplasm KS81H1640HF conditions resistance to biotype D of Hessian fly[10]. Monosomic and ditelosomic stocks of the cultivars Wichita and Chinese Spring are known to be susceptible. The various F_2 and F_3 progenies were scored for *H13* gene by infestation with Hessian fly in a greenhouse. Resistant plants indicated the presence of the *H13* gene. Methods of infestation and of determining resistance or susceptibility of individual seedlings were similar to those described by Cartwright and LaHue[2]. Adult Hessian flies were allowed to oviposit for two days on seedlings in the one-leaf stage. Plants were then examined for eggs and found to be infested with 10–15 eggs per plant. After infestation, all plants were grown in a controlled climate chamber maintained on a 12-hour photophase and at 20 ± 1° C. Plant reaction was determined about 15 days after infestation. Susceptible plants were stunted, dark green, and contained live larvae. In contrast, resistant plants were not stunted, were yellowish green, and had large numbers of dead larvae.

Wichita monosomics used in chromosome location studies were kindly provided by Dr. R. Morris. The homozygous *H13H13* germplasm KS81H1640HF was derived from F_3 lines of a cross between a synthetic hexaploid wheat (KU-221-14)/Eagle//NE73640/Cheney[13]. A selection from this germplasm hereafter designated KS81H1640HF was used in cytogenetic mapping. Since it was known that the *H13* gene was derived from *A. squarrosa*, the D-genome donor of common wheat, only D-genome Wichita monosomics were used in the crosses. Seed of the seven D-genome monosomics (1D to 7D) were germinated, $2n = 41$ chromosome plants identified by root tip counts, and crossed with KS81H1640HF. Monosomic ($2n = 41$) hybrid plants were identified and allowed to self. The F_2 plants from the seven monosomic families were scored for *H13* gene segregation. The resistant F_2 plants were selfed to obtain F_3 seed and again scored for *H13* gene segregation.

Once the chromosomal location of *H13* was established on 6D, crosses of KS81H1640HF were made with Chinese Spring 6Dp (short arm) and 6Dq (long arm) ditelosomic lines that were kindly provided by Dr. E. R. Sears. The F_1 monotelodisomic plants were allowed to self and F_2 seed were harvested. Sixty F_2 plants from each family were scored for their chromosome constitution and *H13* gene segregation. Techniques used in root tip chromosome counts and N-banding analysis have been published[3].

Results and Discussion

Monosomic analysis is designed to locate a gene on a specific chromosome[14]. If the gene is not located on a monosomic chromosome, F_2 plants will give a disomic ratio. Conversely, if the gene is located on a monosomic chromosome, the F_2 plants will deviate significantly from a disomic ratio. In the present study, six of the seven monosomic F_2 families (1D to 5D and 7D) gave a 3:1 segregation for the *H13* gene, an indication that the gene was not located on these chromosomes (Table I). The critical monosomic 6D family gave a large excess of resistant plants and deviated significantly from a 3:1 ratio, suggesting that *H13* is located on chromosome 6D. Most of the susceptible plants, presumably, were nullisomics. Root tip analysis was not possible on a majority of the susceptible plants as they were weak and stunted, and died soon thereafter. However, chromosome counts were made on two plants and they were nullisomic ($2n = 40$).

The resistant F_2 plants from all monosomic families were allowed to self and F_3 families were tested for the *H13* gene. Six, noncritical, monosomic, F_3 families produced resistant to segregating progenies in a ratio of 1:2. The critical monosomic family, 6D, which deviated significantly and gave mostly resistant progenies (Table II), provided additional support for the location of the *H13* gene on chromosome 6D. Among the 83 resistant 6D F_3 families, 35 produced no susceptible plants, and the other 48 produced a few susceptible plants (ca. 10 percent) as a result of nullisomy, but were classified as resistant families. The occurrence of 13 monosomic 6D F_3 families that produced an excessive number of susceptible plants was unexpected. Some preliminary data and reasons for this discrepancy are discussed later.

For arm location and linkage mapping analysis of the *H13* gene with the centromere, F_2 progenies derived from crosses of the 6Dp and 6Dq telosomic stocks with KS81H1640HF germplasm were analyzed for their chromosome constitution and reaction to Hessian fly. Based on the functioning of 21 and $21t$ ($20 + t$) gametes in the F_1 plant, F_2 plants with the chromosome constitution 42, $42t$ ($41 + t$), and $42tt$ ($40 + 2t$) are expected. Plants with different chromosome constitutions, however, were also encountered and must have arisen from meiotic abnormalities. These plants were not included in mapping analysis data (Tables III and IV). Another expectation is that, depending upon the distance of the gene from the centromere, certain recombinants will be expected for the arm on which the gene is located, and none for the arm lacking the gene.

The monotelodisomic 6Dp F_2 gave 58 resistant and two susceptible plants (Table III). This indicated that the *H13* gene is not

Table I. Segregation for *H13* gene to Hessian fly biotype D in F_2 plants from crosses involving Wichita monosomics and KS81H1640HF germplasm

Monosomic chromosome	No. F_2 plants resistant	susceptible	χ^2 (3:1)
1D	74	26	0.05
2D	55	17	0.08
3D	76	32	1.24
4D	73	20	0.63
5D	63	18	0.35
6D	258	24	40.89**
7D	78	20	1.11
Total (excluding 6D)	419	133	0.24

** Highly significant at $P < 0.01$; all other values not significant

Table II. Distribution of F_3 lines of Wichita monosomic/KS81H1640HF germplasm crosses for reaction to Hessian fly

Monosomic chromosome	No. F_3 lines resistant	segregating	χ^2 (1:2)
1D	5	14	0.40
2D	7	13	0.02
3D	10	16	0.30
4D	6	6	1.50
5D	2	13	2.70
6D	83	13	121.92**
7D	5	8	0.18
Total (excluding 6D)	35	70	0.00

** Highly significant at $P < 0.01$; all other values not significant

Table III. Segregation of the *H13* gene in F_2 plants of monotelodisomic 6Dp hybrid heterozygous for 6Dp and the *H13* gene

Somatic chromosome number	Reaction and no. plants resistant	susceptible
$2n = 42$	29	0
$2n = 42t$	23	1
$2n = 42tt$	1	1
$2n = 41t$	2	0
$2n = 41$	3	0
Total	58	2

located on the short arm. Two exceptional plants were encountered, a 42*t* plant with a susceptible reaction and a 42*tt* plant with a resistant reaction. The 42*t* plant must not carry a 6D chromosome bearing the *H13* gene. The 42*tt* resistant plant either contains one telosomic 6Dq derived from a 6D chromosome, or the 6D chromosome is present but is missing another chromosome. The latter explanation also would account for the two 41*t* resistant plants.

The 6Dq F_2 family gave 46 resistant plants and 13 susceptible plants (Table IV). The data clearly indicated that the *H13* gene is located on the 6Dq arm and is not tightly linked with the centromere. The calculation of map distance of the gene from the centromere is complicated because of the unequal male and female transmission of the telocentric chromosome. Transmission through the female of the normal chromosome and the 6Dq telocentric homologue was equal since the frequencies fitted a 1:1 ratio, i.e., 26(42 and 42*tt* chromosome plants):25 (42*t* chromosome plants) (Table IV). Therefore, the transmission rate, *p*, for the telocentric 6Dq through the male was estimated from 42*tt* plants and was 5/26 or 19.2 percent. The recombination between the gene and the centromere is calculated by the maximum likelihood method[1,22]. From these calculations, genetic distance between the *H13* gene and the centromere was found to be 35.0 ± 8.0 recombination units.

As discussed earlier, F_3 families derived from monosomic 6D F_2 resistant plants produced a greater number of susceptible plants than would be expected for nullisomy and were classified as segregating (Table II). It was assumed that some of these plants must have arisen because of cytological instability and may have lost their 6D chromosome(s). Five susceptible plants from one of these F_3 families were analyzed by N-banding. In the common wheat cv. Wichita, there are 14 pairs of banded chromosomes: all B genome chromosomes, all A genome chromosomes excluding 1A and 6A, and chromosomes 2D and 7D[3]. Thus, because the 6D chromosome cannot be identified, the status of the total number of banded and unbanded chromosomes was determined.

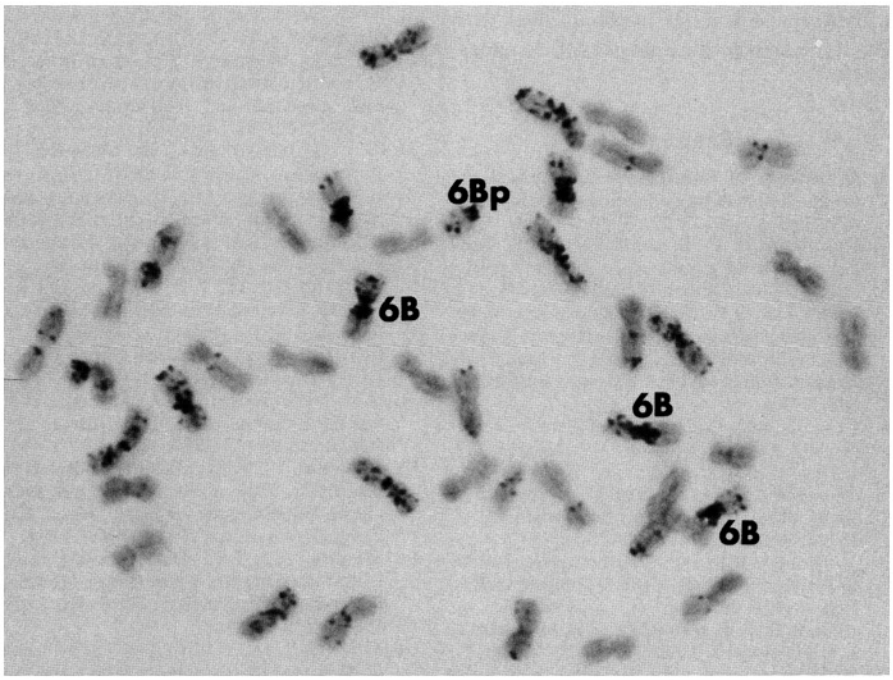

FIGURE 1 N-banded somatic chromosomes at metaphase in a susceptible plant 82-6-1-19-3-1 derived from an F_3 monosomic 6D family that segregated for an excess of susceptible plants to Hessian fly. There are 12 unbanded chromosomes and 30*t* banded chromosomes. The plant was trisomic for 6B and telosomic for 6Bp.

As is apparent from the data in Table V and Figure 1, only 11–12 unbanded chromosomes were present in the five susceptible plants analyzed, indicating the plants were nullisomic for 6D. Moreover, there was an extra banded chromosome in all plants, as three plants appeared to be trisomic for 6B, one for 3A, and one for 7D (Table V). One trisomic 6B plant also carried a telosomic chromosome 6Bp (Figure 1). These data demonstrated cytological instability that would lead to loss of 6D chromosomes and other abnormal chromosome behavior in some F_2 plants. Thus, at least 13.5 percent of the F_2 plants must have been cytologically unstable and these produced 13 segregating F_3 families.

In conclusion, it has been unequivocally shown that the *H13* gene is located on 6Dq arm at 35 recombination units from the centromere. This is the first Hessian fly-resistance gene mapped on a D genome chromosome. A certain level of cytological instability was encountered in a fraction of the monosomic families. Therefore, it is advisable to undertake several cycles of backcrossing to bring the transferred gene into a genet-

Table IV. Segregation of the *H13* gene in F_2 plants of monotelodisomic 6Dq hybrid heterozygous for 6Dq and the *H13* gene

Somatic chromosome number	Reaction and no. plants resistant	susceptible
$2n = 42$	20	1
$2n = 42t$	17	8
$2n = 42tt$	4	1
$2n = 41t$	2	1
$2n = 41$	1	1
$2n = 43t$	1	1
$2n = 43$	1	0
Total	46	13

Table V. Chromosome banding analysis of susceptible plants from monosomic 6D F_3 families segregating for an excess of susceptible plants in reaction to Hessian fly

Plant no.	2*n*	No. banded chrom.	No. unbanded chrom.	Other abnormalities
82-6-1-19-1-2	40	29	11	trisomic 3A
82-6-1-19-2-1	40	29	11	trisomic 6B
82-6-1-19-3-1	42t	30t	12	trisomic 6B, telosomic 6Bp
82-6-6-13-1-2	41	29	12	trisomic 7D
82-6-6-13-2-1	40	29	11	trisomic 6B

ic background similiar to the cultivar with which the monosomic analysis will be undertaken.

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