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Transfer of Hessian fly resistance from 'Chaupon' rye to hexaploid wheat via a *2BS/2RL* wheat-rye chromosome translocation *

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Summary. Four wheat-rye lines derived from a cross between hexaploid wheat 'ND 7532' and 'Chaupon' rye were homogeneous for resistance to biotype L of the Hessian fly, *Mayetiola destructor*. Because the wheat parent was susceptible and the rye parent was resistant to larval feeding, resistance was derived from rye. Resistance of 'Chaupon' and the wheat-rye lines was expressed as larval antibiosis. First-instar larvae died after feeding on plants. Chromosomal analyses using C- and N-banding techniques were performed on plants of each line to identify genomes and structural changes of chromosomes. Results showed that two of the resistant lines were chromosome addition lines carrying either the complete rye chromosome, *2R*, or only the long arm of *2R*. The other two resistant lines were identified as being *2BS/2RL* wheat-rye translocation lines. It was concluded, therefore, that the long arm of rye chromosome *2R* carries a gene or gene complex that conditions antibiosis to Hessian fly larvae and, in the *2BS/2RL* translocation lines, this rye chromatin is cytologically stable and can be used directly in wheat breeding programs.

Key words: Hessian fly resistance – Insect antibiosis – Wheat-rye hybrids – C-banding

Introduction

The Hessian fly, *Mayetiola destructor*, is a destructive insect of wheat in many parts of the world. In the USA, genetic resistance in wheat (*Triticum aestivum* L. em Thell.) cultivars has provided control of the insect for the last 30 years. Nineteen major genes that confer resistance to larvae have been identified in *Triticum* spp. and are being used in breeding resistant cultivars (Gallun 1977, for review; Hatchett et al. 1981; Stebbins et al. 1982, 1983; Oellermann et al. 1983; Mass et al. 1987, 1989; Patterson et al. 1988; Obanni et al. 1988, 1989). However, the genetic interaction between wheat and the Hessian fly is highly specific; a gene-for-gene relationship has been demonstrated between resistance in the host and avirulence in the insect (Hatchett and Gallun 1970). Eight biotypes of the Hessian fly have been identified from field populations in the USA and are designated Great Plains, A, B, C, D, E, J, and L (Gallun 1977, for review; Sosa 1981). These biotypes differ in their avirulence/virulence to wheats carring resistance genes *H3, H5, H6,* or *H7H8*. Because of the genetic variability for host-specific virulence present in the Hessian fly, diverse sources of resistance in wheat and its relatives are continually being sought.

Rye, *Secale cereale* L., is an important source of disease and pest resistance genes for improvement of cultivated wheat (Riley and Macer 1966; Zeller and Hsam 1983). However, so far only the short arm of rye chromosome *1R*, carrying the resistance genes *Yr9, Lr26, Sr31,* and *Pm8* against the wheat pathogens stripe rust, (*Puccinia striiformis* West), leaf rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici*), stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.), and powdery mildew (*Erysiphe graminis* DC. f. sp. *tritici* E. Marchal), respectively, has been widely used in cultivars, either in form of a *1RS/1BL* (Zeller 1973; Mettin et al. 1973) or as a *1RS/1AL* translocation (Sebesta and Wood 1978; Zeller and Fuchs 1983). Although resistance to Hessian fly in rye has been known for many years (Painter 1951), there has

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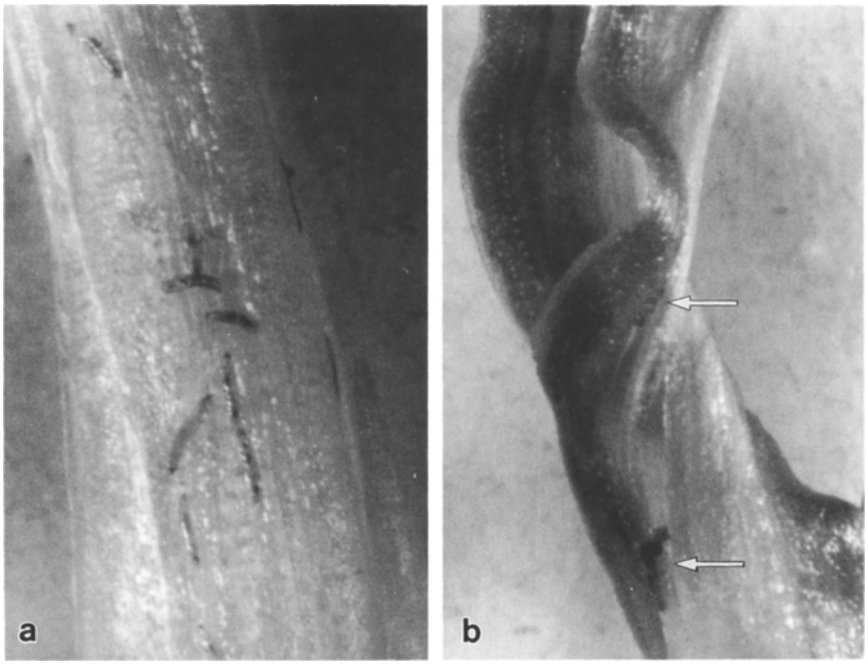

Fig. 1 a and b. Resistance to Hessian fly in wheat-rye line KSWR 69-2-4-3 showing: **a** dead first-instar larvae on second-leaf sheath (first-leaf sheath has been removed to expose larvae), and **b** chlorotic lesions where larvae had previously fed on second-leaf sheath. Note dead larvae (*arrows*) that remained affixed to lesions

been little effort to ulilize rye genes in the development of resistant wheat cultivars. This paper describes resistance to Hessian fly in 'Chaupon' rye and its transfer to the hexaploid wheat genome via either a spontaneous or tissue culture-induced *2BS/2RL* wheat-rye chromosome translocation.

Materials and methods

Plant material

Four wheat-rye (WR) lines, derived from a cross between hexaploid wheat 'ND 7532' (Froid × Centurk) and diploid rye 'Chaupon', were used in this study. 'ND 7532' is susceptible and 'Chaupon' is resistant to Hessian fly. The WR lines originated from plants regenerated from scutellar calli of hybrid embryos. The plants were backcrossed to 'ND 7532' after colchicine-induced chromosome doubling. The production of this plant material has been described in detail by Lapitan et al. (1984). Three of the lines, KSWR 297-1-1-9, KSWR 78-2-2-5, and KSWR 69-2-4-3, are F_5 progenies of the cross [(ND 7532 × Chaupon) × 4 * ND 7532]. The fourth line, KS85HF 011-5, is an F_3 progeny of the cross [(ND 7532 × Chaupon × 4 * ND 7532) × Karl]. 'Karl' is a Hessian fly susceptible common wheat cultivar. The WR lines were previously tested for resistance in the BC_4F_3 generation, and all were homogeneously resistant (J. H. Hatchett and R. G. Sears, unpublished data).

Resistance evaluations

The four WR lines, 'ND 7532', and 'Chaupon' were evaluated in the seedling stage for reaction to biotype L Hessian fly in a greenhouse. The WR lines and the wheat and rye parents were tested in two separate experiments. Biotype L is the most virulent biotype presently found in the field; larvae can infest wheats carrying *H1H2, H3, H4, H5, H6, H7H8, H11,* and *H15*, but cannot infest wheats carrying *H9, H10, H12, H13, H14,* or *H16* through *H19*. Seeds of each WR line or the parents were seeded in rows in wooden flats containing soil. The number of plants tested varied, depending on the number of seeds available and their level of germination. Greenhouse temperatures were maintained between 18° and 24 °C throughout the tests.

Methods of infestation and testing for resistance were reported previously (Hatchett et al. 1981). Adult Hessian flies were allowed to oviposit for 8 h on plants in the one-leaf stage. Plants were examined after oviposition and all were found to be infested with large numbers of eggs on the first leaf. Susceptible and resistant plant reactions were determined 15 days after egg infestation. All plants were examined under a stereoscopic microscope (20 ×) for presence of larvae on the second leaf sheath. The condition of the larvae, either live or dead, was also recorded.

Chromosomal analyses

Chromosome identification was carried out by using the Giemsa C-banding technique described by Giraldez et al. (1979). The N-banding procedure used was the same as described by Endo and Gill (1983, 1984). Microphotographs were taken with a Zeiss Photomicroscope III using a Kodak Imagecapture AHU microfilm 5460.

Results and discussion

Resistance evaluations and characterization

The WR lines and 'Chaupon' rye were homogeneously resistant, and 'ND 7532' wheat was homogeneously susceptible to biotype L Hessian fly, confirming that resistance was derived from the rye parent (Table 1). All plants of the four WR lines and 'Chaupon' were highly antibiotic and contained large numbers of dead first-instar larvae (Fig. 1 a). Dead larvae retained the normal red body color of 1- to 3-day-old first instars and were

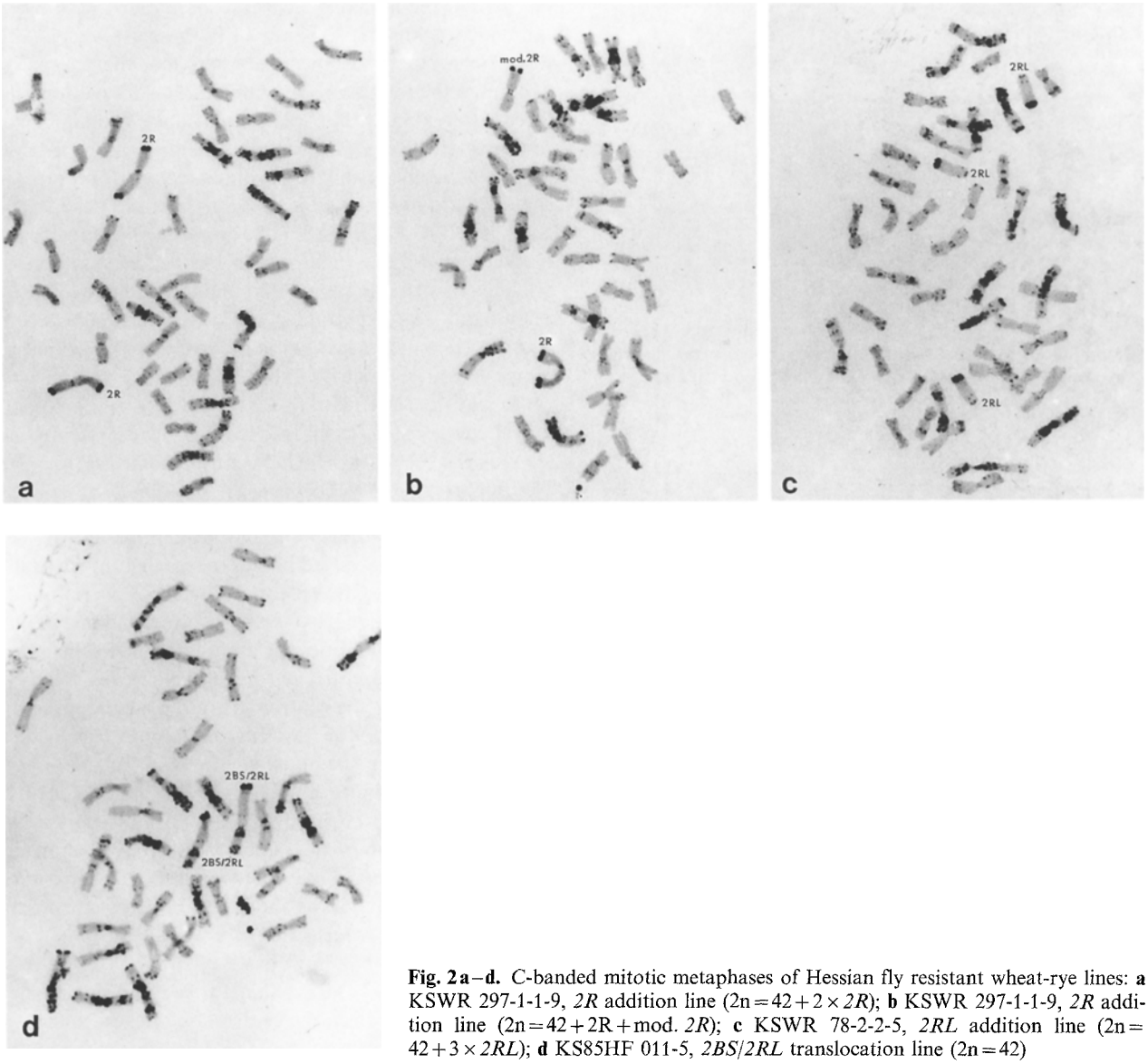

Fig. 2a–d. C-banded mitotic metaphases of Hessian fly resistant wheat-rye lines: **a** KSWR 297-1-1-9, *2R* addition line ($2n=42+2\times 2R$); **b** KSWR 297-1-1-9, *2R* addition line ($2n=42+2R+$mod. *2R*); **c** KSWR 78-2-2-5, *2RL* addition line ($2n=42+3\times 2RL$); **d** KS85HF 011-5, *2BS/2RL* translocation line ($2n=42$)

about 0.5 mm long, whereas larvae on 'ND 7532' were late second instars, translucent white, and 3.0–4.5 mm long. At 15 days after infestation, plants of the WR lines and 'Chaupon' had developed to the third-leaf stage, showed no symptoms of stunting, and retained their normal green color. In contrast, 'ND 7532' plants developed only to the two-leaf stage, were severely stunted, and the leaves were dark blue-green in color.

Most of the dead larvae on resistant plants were found 1–4 cm above the base of the second-leaf sheath, indicating that the larvae were carried upward from the base as the second-leaf initial elongated. Resistant plants also developed pronounced chlorotic lesions on the abaxial surface of the second leaf (Fig. 1 b). These lesions were void of chlorophyll and often had dead larvae affixed to the epidermis, indicating that larvae fed before dying. Thus, it is conceivable that the lesions developed as a result of hypersensitive reactions to larval feeding which, in turn, prevented establishment and development of first-instar larvae.

Chromosomal analyses

As shown in Fig. 2, C-banding of mitotic metaphases permitted the identification of rye chromatin present in the four WR lines. KSWR 297-1-1-9 showed a chromosome number of $2n=43$ or 44 and carried one or a chromosome pair of rye chromosomes in addition to the normal chromosome complement of wheat. In 2 of 19 plants analyzed, the added rye chromosome was as a monosomic addition, whereas in 17 plants this chromosome was present as a disomic addition. The added chromosome pair of rye can be easily distinguished from the chromosomes of wheat by the presence of large telomeric

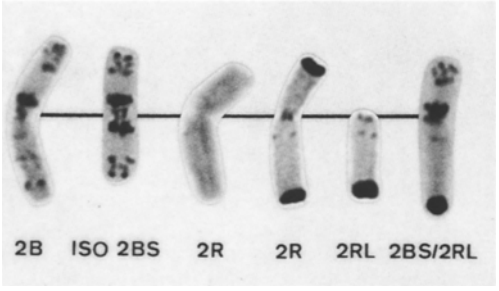

Fig. 3. C- and N-banding patterns of chromosome *2B* of wheat and *2R* of rye present in Hessian fly resistant wheat-rye lines (from left to right): *2B* (C-banding), isochromosome *2BS* (C-banding), *2R* (N-banding), *2R* (C-banding), telocentric chromosome *2RL* (C-banding), *2BS/2RL* translocation (C-banding)

Table 1. Reactions of 'Chaupon' rye and 'ND 7532' wheat and four wheat-rye lines derived from a cross between 'ND 7532' and 'Chaupon' to biotype L Hessian fly

Line or parent	No. of plants	
	Resistant	Susceptible
KSWR 297-1-1-9	18	0
KSWR 78-2-2-5	16	0
KSWR 69-2-4-3	11	0
KS85HF 011-5	23	0
'Chaupon' rye	18	0
'ND 7532' wheat	0	24

C-bands in both arms (Figs. 2a and 3). Further, this rye chromosome shows a small C-band adjacent to the centromere in the long arm, which is characteristic for rye chromosome *2R* (Sybenga 1983; Schlegel et al. 1986).

The N-banding pattern also verified the presence of rye chromosome *2R* in KSWR 297-1-1-9. The added chromosome pair showed only a very faint N-band adjacent to the centromere in the long arm, which corresponds to the band also observed after C-banding in this region (Fig. 3). N-banding is known to produce only three faint bands in rye: two bands adjacent to the centromere in the long arms of chromosomes *2R* and *6R*, and one band adjacent to the centromere in the short arm of chromosome *3R* (Schlegel and Gill 1984). Because the added chromosome of this line presents a faint N-band in the long arm and, based on its morphology and C-banding pattern, rye chromosome *6R* can be excluded, this result confirms that the added chromsome is *2R* of rye.

One plant of KSWR 297-1-1-9 showed an unusual cytological instability. In 28 of 31 metaphases analyzed, the added rye chromosomes presented the characteristic telomeric C-bands in both arms; however, three metaphases were found which were heterozygous for a modified rye chromosome *2R*. The C-banded metaphases shown in Fig. 2a and b were derived from the same plant. In Fig. 2a, the added rye chromosome pair *2R* showed the typical telomeric C-bands in both arms, whereas in Fig. 2b one chromosome of *2R* has lost the telomeric C-band in the long arm. The same deletion was found in three different cells, but was present only in one of two root tips analyzed, indicating that the three modified rye *2R* chromosomes probably originated from one breakage event followed by the loss of the terminal C-heterochromatin. Although similar cytological instability of rye telomeric C-heterochromatin has not been reported previously, it is well known that many wheat-rye hybrids carry modified rye chromosomes which show a reduced amount or complete loss of telomeric C-heterochromatin (Appels 1982; Gustafson 1983).

In addition, one plant of KSWR 297-1-1-9 was found to be heterozygous for an isochromosome *2BS*, containing only the short arm of wheat chromosome *2B* (Fig. 3). The isochromosome *2BS* probably originated by centric breakage and fusion of the sister chromatids.

KSWR 78-2-2-5 varied in chromosome number from $2n=43$ to $2n=45$. Of 25 plants analyzed, 22 were found to be disomic additions, two were trisomic, and one plant was a monosomic addition of a rye telocentric chromosome. The rye telocentric chromosome showed a small C-band adjacent to the centromere and a large telomeric C-band which identifies this chromosome as being the long arm of the rye chromosome *2R* (Fig. 2c).

Twenty-three plants of KSWR 69-2-4-3 and 27 plants of KS85HF 011-5 were analyzed and all showed a chromosome number of $2n=42$. C-banding analysis showed that these two lines were homozygous for a wheat-rye translocation involving the short arm of wheat chromosome *2B* and the long arm of rye chromosome *2R* (Fig. 2d). The C-banding pattern of this *2BS/2RL* translocation (Fig. 3) shows that the break point of the translocation lies within the centromeric region, indicating that this translocation originated by centric breakage and fusion. Previous studies have shown that most wheat-rye translocations are centric fusion products which resulted from misdivision of univalents at meiosis with subsequent fusion of the telocentric chromosomes (Lukaszewski and Gustafson 1983; Friebe and Larter 1988). The breakage fusion cycle translocation may have occurred during the meiotic division prior to culture of embryo explants. Alternatively, the *2BS/2RL* translocation present in KSWR 69-2-4-3 and KS85HF 011-5 could have been also produced during the callus stage of tissue culturing.

Since the wheat parent 'ND 7532' was susceptible and the rye parent 'Chaupon' was resistant to Hessian fly larvae, the resistance expressed in the WR lines has to be derived from the rye parent. Our results showed that all four WR lines derived from that cross carry the long arm of rye chromosome *2R*, either present as a complete chromosome *2R* or as a *2RL* telocentric addition, or in the

form of a *2BS/2RL* wheat-rye translocation. Therefore, the gene or gene complex conditioning larval antibiosis has to be located on the long arm of rye chromosome *2R*.

In KSWR 69-2-4-3 and KS85HF 011-5, this rye segment is present in a cytologically stable form as a *2BS/2RL* translocation compensating for the missing *2BL* arm of wheat. Based on vigor and fertility of lines KSWR-69-2-4-3 and KS85HF 011-5 containing the *2BS/2RL* translocation, it appears that the *2RL* arm not only compensates for the missing *2BL* arm but, in addition, may be exhibiting a heterotic response. Because of Hessian fly resistance and excellent compensation, germ plasm containing the *2BS/2RL* translocation may have significance in breeding and cultivar improvement of wheat. The development of wheat cultivars having the *2BS/2RL* translocation will provide a broader base of genetic resistance to all known biotypes of Hessian fly.

References

Appels R (1982) The molecular cytology of wheat-rye hybrids. Int Rev Cytol 80:83–132

Endo TR, Gill BS (1983) Identification of wheat chromosomes by N-banding. In: Proc 6th Intern Wheat Genet Symp, Kyoto, pp 355–359

Endo TR, Gill BS (1984) Somatic karyotype, heterochromatin distribution and nature of chromosome differentiation in common wheat, *Triticum aestivum* L. em Thell. Chromosoma 89:361–369

Friebe B, Larter EN (1988) Identification of a complete set of isogenic wheat/rye D-genome substitution lines by means of Giemsa C-banding. Theor Appl Genet 76:473–479

Gallun RL (1977) Genetic basis of Hessian fly epidemics. Ann N Y Acad Sci 287:223–229

Giraldez R, Cermeño MC, Orellana J (1979) Comparison of C-banding pattern in the chromosomes of inbred lines and open pollinated varieties of rye, *Secale cereale* L. Z Pflanzenzuecht 83:40–48

Gustafson JP (1983) Cytogenetics of triticale. In: Swaminathan MS, Gupta PK, Sinha U (eds) Cytogenetics of crop plants. McMillan, New York, pp 225–250

Hatchett JH, Gallun RL (1970) Genetics of the ability of the Hessian fly, *Mayetiola destructor*, to survive on wheats having different genes for resistance. Ann Entomol Soc Am 63:1400–1407

Hatchett JH, Martin TJ, Livers RW (1981) Expression and inheritance of resistance to Hessian fly in synthetic hexaploid wheats derived from *Triticum tauschii* (Coss) Schmal. Crop Sci 21:731–734

Lapitan NLV, Sears RG, Gill BS (1984) Translocations and other karyotypic structural changes in wheat × rye hybrids regenerated from tissue culture. Theor Appl Genet 68:547–554

Lukaszewski AJ, Gustafson JP (1983) Translocations and modifications of chromosomes in triticale × wheat hybrids. Theor Appl Genet 64:239–248

Maas FB III, Patterson FL, Foster JE, Hatchett JH (1987) Expression and inheritance of resistance of 'Marquillo' wheat to Hessian fly biotype D. Crop Sci 27:49–52

Maas FB III, Patterson FL, Foster JE, Ohm HW (1989) Expression and inheritance of resistance of ELS 6404-160 durum wheat to Hessian fly. Crop Sci 29:23–28

Mettin P, Blüthner WD, Schlegel G (1973) Additional evidence on spontaneous 1B/1R wheat-rye substitution and translocations. In: Proc 4th Int Wheat Genet Symp, Columbia, pp 179–184

Obanni M, Patterson FL, Foster JE, Ohm HW (1988) Genetic analyses of durum wheat PI 428435 to the Hessian fly. Crop Sci 28:223–226

Obanni M, Ohm HW, Foster JE, Patterson FL (1989) Genetics of resistance of PI 422297 durum wheat to the Hessian fly. Crop Sci 29:249–252

Oellermann CM, Patterson FL, Gallun RL (1983) Inheritance of resistance in Luso wheat to Hessian fly. Crop Sci 23:221–224

Painter RH (1951) Insect resistance in crop plants. MacMillan, New York

Patterson FL, Foster JE, Ohm HW (1988) Gene H16 in wheat for resistance to Hessian fly. Crop Sci 28:562–564

Riley R, Macer RFC (1966) The chromosomal distribution of genetic resistance of rye to wheat pathogens. Can J Genet Cytol 8:640–653

Schlegel R, Gill BS (1984) N-banding analysis of rye chromosomes and the relationship between N-banded and C-banded heterochromatin. Can J Genet Cytol 26:765–769

Schlegel R, Melz G, Mettin D (1986) Rye cytology, cytogenetics and genetics – current status. Theor Appl Genet 72:721–734

Sebesta EE, Wood EA Jr (1978) Transfer of greenbug resistance from rye to wheat with x-rays. Agron Abst 1978:61–62

Sosa O Jr (1981) Biotype J and L of the Hessian fly discovered in an Indiana wheat field. J Econ Entomol 74:180–182

Stebbins NB, Patterson FL, Gallun RL (1982) Interrelationships among wheat genes *H3, H6, H9,* and *H10*, for Hessian fly resistance. Crop Sci 22:1029–1032

Stebbins NB, Patterson FL, Gallun RL (1983) Inheritance of resistance of PI94587 wheat to biotypes B and D of Hessian fly. Crop Sci 23:251–253

Sybenga J (1983) Rye chromosome nomenclature and homoeology relationships. Z Pflanzenzeucht 90:297–304

Zeller FJ (1973) 1B/1R wheat-rye chromosome substitutions and translocations. In: Proc 4th Int Wheat Genet Symp, Columbia, pp 209–211

Zeller FJ, Fuchs S (1983) Cytologie und Krankheitsresistenz einer 1A/1R- und mehrerer 1B/1R-Weizen-Roggen-Translokationssorten. Z Pflanzenzuecht 90:285–296

Zeller FJ, Hsam SLK (1983) Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. In: Proc 6th Int Wheat Genet Symp, Kyoto, pp 167–173