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Deletion Mapping of a Nematode Resistance Gene on Rye Chromosome 6R in Wheat

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ABSTRACT

Four deletion mutants of rye chromosome 6R were identified in progeny of wheat (*Triticum aestivum* L.) lines of *ph1bph1b* genotype and monosomic for chromosome 6R. The rye chromosome carried a resistance gene against the cereal cyst nematode (CCN) (*Heterodera avenae* Woll.) and this chromosome originated in triticale line T-701 (× *Triticosecale* Witt.). The deletion mutants were selected on the basis of dissociation of three isozyme loci on the long arm of the rye (*Secale cereale* L.) chromosome. Three plants and their progeny showed expression of the rye genes α-*Amy-R1* and *Got-R2* but lacked the gene *PgdR2*. The other plant and its progeny showed expression of the α-*Amy-R1* gene while the genes *Got-R2* and *PgdR2* were absent. The four deletion chromosomes displayed long-arm terminal deficiencies of different sizes which enabled mapping of the rye isozyme genes and the CCN resistance gene. The distal to proximal order of the 6R isozyme loci was found to be: *PgdR2*, *Got-R2*, and then α-*Amy-R1*. Bioassay tests demonstrated that the CCN resistance gene (*CreR*) was located on an interstitial section of the long arm of 6R adjacent to *Got-R2*.

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DELETION MUTANTS of wheat chromosomes (Endo, 1995; Endo and Gill, 1996) have proven to be valuable tools for mapping molecular markers and morphological characters to physical segments of chromosomes. By allocating markers to these types of chromosomes (Werner et al., 1992; Hohmann et al., 1994, 1995; Mickelson-Young et al., 1995; Gill et al., 1996a,b) the validity of maps based on conventional recombination studies can be confirmed. In addition, deletion-based maps have the advantage of not requiring complete synapsis and recombination of pairing chromosomes which are essential for conventional mapping analyses. Structural differences between pairing chromosomes can prevent full synapsis and distort segregation ratios (Burnham, 1962) thus hindering recombination analysis. Cytogeneticists find both deletion and recombination maps useful first, for selection of suitable probes for use with homoeologous chromosomes from related species and second, to assist in planning strategies for the efficient transfer of useful genes from alien chromosomes to wheat.

Devos et al. (1993) presented a recombination-based map of the rye chromosomes showing the distribution of molecular markers and a suggested history of the structural rearrangements involving those chromosomes. The rye map showed numerous translocations involving most of the rye chromosomes (with the exception of 1R) as compared with their wheat homoeologues. The complex structure of the rye genome suggests that gene transfer from these chromosomes to wheat by homoeologous recombination may be difficult.

With the exception of rye chromosome 1R (Endo et al., 1994), there are no other known sets of chromosome deletion lines available for rye which could be used to elucidate the structures of those chromosomes further.

Several years before the study of Devos et al. (1993) was published, a project was initiated to transfer a CCN resistance gene on rye chromosome 6R to a wheat chromosome. Fisher (1982) had previously described a line of triticale (T-701) showing high levels of resistance to CCN. Asiedu et al. (1990) found that the resistance character was controlled by a single dominant gene located on rye chromosome arm 6RL of triticale T-701. Homoeologous recombination was chosen as the preferred method of achieving transfer of the CCN gene to wheat because a similar technique had been used successfully by Riley et al. (1968), Sears (1973), and Joshi and Singh (1979) to incorporate alien disease resistance genes into wheat and because Koebner and Shepherd (1985, 1986) and Koebner et al. (1986) had reported the production of recombinants between a rye chromosome and wheat in the absence of the wheat *Ph1* pairing gene.

However, the absence of confirmed recombination between the 6R chromosome segment and homoeologous wheat chromosomes of group 6 in a *ph1bph1b* background was reported earlier (Dundas et al., 1992). Several deletion mutants involving rye chromosome 6R were found (Dundas et al., 1992) and this paper describes their isolation and detailed structure. These deletion stocks may prove useful for further physical mapping of that chromosome and even provide a strategy for future gene transfer from chromosome 6R to a wheat chromosome.

MATERIALS AND METHODS

Genetic Stocks

Crosses were made by Dr. Robert Asiedu (formerly of Waite Agricultural Research Institute, Adelaide) between triticale T-701 and wheat to produce a disomic chromosome substitution line 6R(6D). Dr. Asiedu later crossed this substitution line with Sears' *ph1b* mutant (Sears, 1977) in a 'Chinese Spring' background (Asiedu et al., 1990). F_2 progeny of those plants were screened for the presence of the isozymes produced by the genes *Got-D2* and *Got-R2* located on the long arms of 6D (Hart, 1975) and 6R (Tang and Hart, 1975), respectively, to ensure that both of these chromosomes were present. The monosomic status of chromosome 6R in the F_2 plants was confirmed by C-banding of mitotic squash preparations.

F_2 plants homozygous for the *ph1b* gene were selected on the basis of two criteria, first, for the increased frequency of univalents and rod bivalents at meiosis (Koebner and Shepherd, 1985) and second, for the presence of homoeologous pairing after test crossing with the related species *Aegilops variabilis* Eig (Asiedu et al., 1990). From 10 F_2 plants homozygous for the *ph1b* gene, 2787 progeny seeds were produced. In addition, 999 seeds representing progeny of 12 F_3 plants monosomic for 6R chromosome, which had been derived from five different F_2 families, were produced.

Seed stocks of 'Schomburgk' wheat used in crossing activities with suspected recombinants were kindly provided by Dr. A. Rathjen at the Waite Campus, Adelaide University. The addition line containing the telocentric 6RL chromosome was produced by Dr. R. Asiedu in family B83-9C-136-3-3-12 while the 6RS addition line was isolated in family B84-9C-2-1-7b-791-5-7.

Isozyme Screening and Maintenance of Stocks

Detection of changes to the structure of rye chromosome 6R was based on the dissociation of rye isozyme markers α-*Amy-R1*, *Got-R2*, and *PgdR2*, previously traced to the long arm of chromosome 6R by Ainsworth et al. (1987), Tang and Hart (1975), and Salinas and Benito (1983), respectively.

To determine isozyme phenotypes, seeds were surface sterilized in a 12% (v/v) sodium hypochlorite solution for 2 min, rinsed thoroughly with water, and germinated on moist filter paper at about 25°C. After 4 d, extracts were taken from the endosperm of each seedling and analyzed for the isozymes of α-amylase (E.C. 3.2.1.1) and 6-phosphogluconate dehydrogenase (6-PGD) (E.C. 1.1.1.44). Two days later, extracts were taken from green leaf tissue and tested for glutamate oxaloacetate transaminase (GOT) (E.C. 2.6.1.1). The isozymes of α-amylase were resolved on flat bed IEF gels following the technique of Ainsworth et al. (1987) and the gels scored for a band controlled by the α-*Amy-R1* locus. The isozymes of 6-PGD and GOT were separated by discontinuous polyacrylamide slab system of Rao and Rao (1980) and Hart (1975), respectively, and unique bands controlled by *PgdR2* and *Got-R2* loci identified.

Seedlings suspected of containing 6R-wheat recombinants were transplanted to the glasshouse and crossed with wheat Schomburgk as the male parent. Progeny tests on either selfed or backcrossed F_1 seedlings from suspected recombinants were conducted to confirm the isozyme patterns of the parent plant.

Chromosome Studies

Actively growing root tips were collected from 4-d-old seedlings and pretreated for 24 h in iced water. Root tips were fixed in freshly prepared 1:3 acetic ethanol and stored until required at −10°C. Squash preparations were made after softening the root tips in 45% (v/v) acetic acid for 10 min at room temperature. Dried slides were C-banded by the method of Koebner and Shepherd (1985). Before examination, slides were made permanent by mounting the coverslips on immersion oil. Dried metaphase I chromosome spreads were C-banded following the method for mitotic chromosomes of Koebner and Shepherd (1985) with the exception that denaturing of slides was performed for 15 min in a 5% (v/v) $Ba(OH)_2$ solution at room temperature (about 25°C).

Nematode Bioassays

The assay method of Fisher (1982) was conducted in three stages and control plants of Schomburgk and Chinese Spring (susceptible) and substitution 6R(6D) (resistant) were included in each bioassay. Families with confirmed dissociations of 6RL markers or control plants of 6RL addition stock were screened for the presence or absence of isozymes of α-*Amy-R1*. After several weeks, leaf samples were taken for determination of the presence of *Got-R2* isozymes. Wheat plants containing the short arm telocentric 6RS chromosome were selected using the dot-blot method of Rogowsky et al. (1991) and employing the rye-specific probe pAW161 (Guidet et al. 1991). Statistical tests for significant differences between means for the genotypes in the bioassay tests were conducted.

RESULTS

Isozyme Dissociations

A total of 3786 wheat seedlings from plants which had undergone either one or two meiotic cycles in a homozygous *ph1b* background were screened for disso-

ciation between three rye isozymes located on the long arm of chromosome 6R. About 291 seedlings showing apparent dissociation of the three marker isozymes were isolated from the above stocks and 172 of these plants were progeny tested, the remainder either failing to survive to maturity or being sterile. Several plants were found with confirmed dissociations of isozyme markers.

Three of the confirmed dissociation plants and their progeny possessed the rye isozyme genes α-*Amy-R1* and *Got-R2* but lacked the gene *PgdR2*. The fourth plant and its progeny had the α-*Amy-R1* gene but lacked the other two isozymes. Cytological examination showed that all four dissociation lines had retained the short-arm telomeric knob of 6R chromosome but had lost terminal segments of the long arm.

Morphology of Chromosome 6R and Deletion Chromosomes

Figures 1 and 2 show the normal C-banded staining pattern of rye chromosome 6R from triticale T-701. The long arm of 6R displays a faintly staining telomeric band, two more intensely staining bands proximal to the telomere and three less deeply staining pairs of dots on the interstitial segment (Fig. 1a, d and d′, e and e′). The short arm displays an intensely staining telomeric knob which is separated from the remainder of the arm by a secondary constriction. A pair of small interstitial dots occur about halfway between the centromere and the distal tip of the short arm (Fig. 1a, d and d′, f and f′).

The C-bands of chromosome 6R have been classified according to a system based on the International System for Human Chromosome Nomenclature (ISCN, 1978). The short arm of the present 6R has been assigned to a single region with bands numbered consecutively from the centromere towards the telomere. The long arm of 6R chromosome has been divided into two regions, with the most intensely staining C-band on that arm as the landmark. Bands of these two regions are numbered consecutively from proximal to distal. Measurements were taken of distances of C-bands from the centromere on several 6R chromosomes and are expressed as ratios to the length of the short arm of the chromosome under study (Table 1). For example, the distance from the centromere to the long-arm telomere of chromosome 6R (band 6RL25) is about 1.48 times the average length of the short arm of that chromosome.

Each deletion chromosome has been classified according to the band in which the breakpoint occurred (Figure 2, Table 1).

Type 6RL22 [del(6R)Ster→::S3→L22]

This chromosome was found in progeny of plant 657 of family B84-9C-2-1-7b after one meiotic cycle with *ph1bph1b* genotype. The distal segment of the long arm carrying the telomeric and subtelomeric C-bands is deleted from this chromosome (Fig. 1b, g and g′). The breakpoint occurs just distal to band 6RL21 (Fig. 2) so that a small amount of band 6RL22 is visible. As well as the long-arm deletion, a segment of the short-arm band 6RS4 between the telomere and the interstitial band 6R53 also appears to have been lost. The evidence for the deletion of a segment of the short arm is two-fold. First, the interstitial C-band on the short arm appears to have been repositioned immediately proximal to the telomere (Fig. 1g and g′). Second, the reduced length of the short arm of this chromosome is reflected in the higher than normal values for the positions of the long arm C-bands in Table 1. The remaining section of the long arm appears to be unchanged.

Type 6RL21 [del(6R)Ster→L21:]

This chromosome was found in plant 791 of the same family as Deletion Type 6RL22 (above). The distal segment of the long arm carrying the telomeric and subterminal C-bands as well as most of band 6RL21 has been lost (Fig. 1c, h and h′). The positions of the remaining C-bands on the long and short arms correspond closely to those of the normal 6R chromosome (Table 1).

Type 6RL1807 [del(6R)Ster→L1807:]

This chromosome was found in plant 54 of family B84-9C-2-2-3-1411 after two meiotic cycles with *ph1bph1b*. The segment carrying the telomeric and two most distal C-bands on the long arm is deleted from this chromosome leaving the long and short arms of nearly the same length (Fig. 1i and i′, Table 1). The breakpoint on this chromosome occurs through band 6RL18 at a point such that approximately 70% of the proximal portion of this band is retained. The positions of the remaining C-bands on the long and short arms correspond closely to those of a normal 6R chromosome (Table 1).

Type 6RL1801 [del(6R)Ster→L1801]

This chromosome was found in progeny of plant 95 of the same family as Deletion Type 6RL1807 (above). The segment carrying the telomeric and two distal C-bands on the long arm is deleted from this chromosome reducing the length of that arm to less than that of the short arm (Fig. 1j and j′, Table 1). The breakpoint on this chromosome occurs through band 6RL18 at a point such that approximately 10% of the proximal portion of this band is retained. The positions of the remaining C-bands on the long and short arms correspond closely to those of a normal 6R chromosome (Table 1).

Meiotic Behavior and Transmission

Metaphase I spreads of pollen mother cells from all of the deletion lines (now of Ph^- genotype) were examined to determine if any wheat chromatin had been incorporated into the deletion chromosomes as indicated by pairing between the deletion chromosome and wheat chromosomes. Forty-five cells of deletion line 6RL22, 61 cells of deletion line 6RL21, 93 cells of deletion line 6RL1807 (Fig. 1k), and 41 cells of line 6RL1801 were checked. In all meiocytes, selected because the deletion chromosome carrying the intensely staining 6R short arm telomere could be seen clearly, that rye chro-

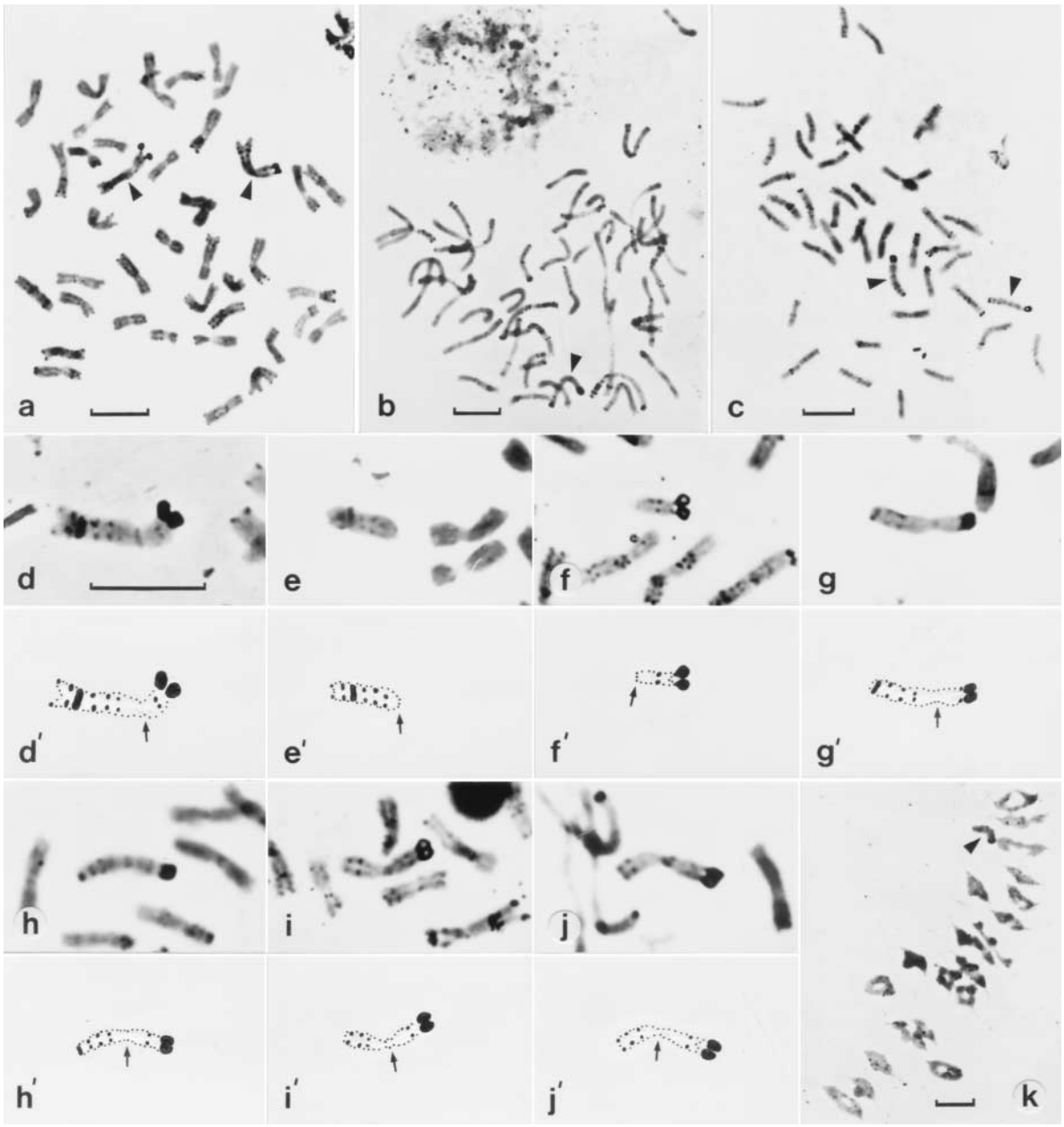

Fig. 1. Photomicrographs and drawings of C-banded preparations of a normal 6R chromosome from triticale T-701 and four deletion mutants in a wheat background. (a) disomic substitution line of normal chromosome 6R(6D) showing 2*n* = 42 chromosomes, (b) monosomic addition line of deletion chromosome 6RL22 showing 2*n* = 43 chromosomes, (c) disomic substitution line of deletion chromosome 6RL21 (6D) showing 2*n* = 42 chromosomes, (d) and (d′) normal 6R chromosome showing prominent short arm telomere and interstitial C-bands on the long arm, (e) and (e′) telocentric 6RL chromosome, (f) and (f′) telocentric 6RS chromosome, (g) and (g′) deletion chromosome 6RL22, (h) and (h′) deletion chromosome 6RL21, (i) and (i′) deletion chromosome 6RL1807, (j) and (j′) deletion chromosome 6RL1801 and (k) metaphase I spread with monosomic addition line of deletion chromosome 6RL1807 showing 21 wheat bivalents and the deletion chromosome as a univalent. Note that Fig. (a) to (c) show whole cell spreads with intact mitotic wheat chromosomes indicating that the 6R deletion mutants did not arise from chromosome breakage during slide preparation. In Fig. (a) to (c) and (k), arrowheads denote 6R chromatin and in Fig. (d′) to (j′), the small arrows indicate the approximate positions of the centromeres. Bars represent 10 μm with Fig. (d) to (j) being of the same magnification.

mosome occurred as a univalent and was never observed to pair with any wheat chromosome (Fig. 1k).

The transmission rates of the normal 6R and deletion mutants of 6R were low. Female transmission, estimated after crosses of *ph1bph1b* plants monosomic for the deletion chromosome with wheat Schomburgk, were as follows: whole 6R chromosome—26% of 474 seedlings over 18 families, deletion line 6RL22—17% of 6 seedlings of one family, deletion line 6RL21—14% of 21 seedlings of two families, deletion line 6RL1807—28% of 32 seedlings of one family, and deletion line 6RL1801—33% of 16 seedlings of one family.

Nematode Bioassays

Control plants with the whole 6R chromosome had few or no nematode females per plant while susceptible

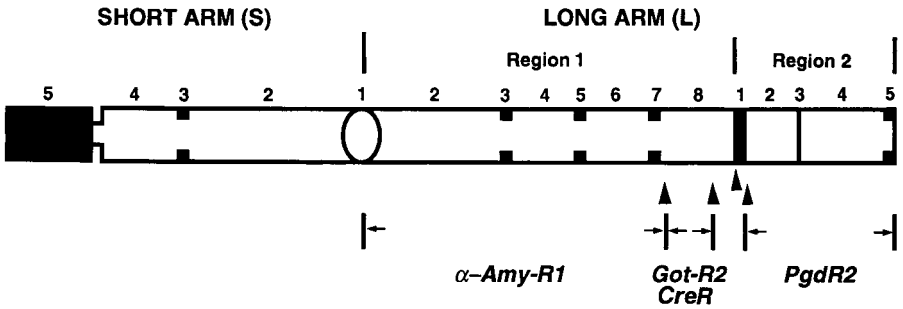

Fig. 2. Idiogram of rye chromosome 6R of triticale T-701 showing the locations of isozyme loci α-*Amy-R1*, *Got-R2*, and *PgdR2* and a nematode resistance gene *CreR* (lower) with respect to C-bands and band nomenclature (upper) Vertical arrowheads denote approximate sites of breakpoints on the long arm resulting in four different deletion mutants of chromosome 6R. Arm lengths and positions of C-bands were determined from measurements given in Table 1.

control plants of Schomburgk and Chinese Spring had significantly higher numbers of nematode females present (Table 2). Plants containing the telocentric 6RL were resistant to the nematode as compared with sib plants without the 6RL chromosome ($P = 0.009$) while those carrying the 6RS telocentric chromosome were susceptible as compared to sib plants without that chromosome ($P = 0.186$). Plants with the deletion chromosome types 6RL22, 6RL21 and 6RL1807 showed significantly low levels of nematode female development as compared to sib plants from the same families lacking that rye segment ($P = 0.044$, 0.029, 0.042, respectively). However, plants with the deletion chromosome type 6RL1801 showed high numbers of nematode females comparable to the susceptible controls ($P = 0.06$) (Table 2).

DISCUSSION

Screening for Wheat-Rye Recombination

The present study set out to isolate rare recombinants between rye chromosome 6R and wheat chromosomes in the attempt to transfer a gene for nematode resistance from the long arm of 6R into wheat by means of Sears' *ph1b* mutant. The Chinese Spring *ph1b* mutant acts by allowing wheat chromosomes to pair and recombine with those of related species (Sears, 1977). No wheat-rye recombinant chromosomes were found, unlike in the earlier pairing studies involving rye chromosome 1R (Koebner and Shepherd, 1985, 1986; Koebner et al., 1986). In the absence of the wheat *Ph1* pairing gene, either through nullisomy for 5B or the presence of the *ph1b* gene, increased meiotic associations have been observed between wheat and rye chromosomes but still at a low level (Upadhya and Swaminathan, 1963; Lacadena, 1967; Dhaliwal et al., 1977; Hutchinson et al., 1983; Schnaider and Priilinn, 1984; Jouve and Giorgi, 1986; Wu et al., 1989; Naranjo and Fernández-Rueda, 1991; Cuadrado et al., 1997). Given that the homoeologous relationship between rye chromosome 6R and chromosomes 6A, 6B, and 6D of wheat is well established (Gupta, 1971), the question arises as to why no wheat-rye recombinants were recovered in the present study. Despite the reports of pairing between rye chromosome 6R and wheat (above), we can find no published papers where wheat-6R recombinant chromosomes have actually been isolated.

Differences in the pairing behavior and structural arrangement of rye chromosomes 1R and 6R could account for the differing rates of recovery of recombinants. First, Naranjo (1982) reported much higher rates of pairing of 1R compared with other rye chromosomes with wheat chromosomes in plants without the normal *Ph1* pairing gene. Second, Riley and Kimber (1966) reported no pairing between the long arm of chromosome II of rye, since designated as 6R (Gupta, 1971), in a nulli-5B wheat missing the *Ph1* pairing gene. Third, molecular marker studies (Benito et al., 1991; Devos et al., 1993) and chromosome pairing studies (Naranjo and Fernández-Rueda, 1991, 1996; Cuadrado et al., 1997) show that the distal regions of 6R chromosome consist of 7R and 3R chromosome segments whereas 1R chromosome shows closer colinearity with wheat group 1 chromosomes. As recombination in cereal chromosomes has been reported to occur at higher levels at the distal regions of the chromosomes (Lukaszewski and Curtis, 1993), homoeologous crossing-over occurring between chromosome 6R in the present study and wheat chromosomes would most likely have involved group 7 or group 3 chromatin and rarely involved chromosome sections carrying the group 6 markers in this study. A strategy to circumvent this difficulty is discussed later.

Origin of 6R Mutants

Apparent spontaneous breakage of cereal chromosomes has been previously reported. Rye chromosomes showing loss of telomeric C-bands have been described by Gustafson et al. (1983) and Dille and Gustafson (1990), and the deletion of a larger segment of the long arm of 6R by Friebe and Larter (1988). In wheat, the occurrence of deletion chromosomes has been associated with the presence of alien chromosomes of *Aegilops* or *Triticum* spp. (Finch et al., 1984; Tsujimoto and Tsunewaki, 1985; Kota and Dvorak, 1988; Endo, 1988, 1995; Tsujimoto and Noda, 1988; Endo and Gill, 1996) although the cause is unknown.

The long-term stability of the present 6R deletion chromosomes is unknown at this stage. While transmission of the deletion chromosomes to progeny is not a cause for concern, previous studies with *Drosophila*

Table 1. Position of C-bands and long arm deletion breakpoints on rye chromosome 6R as shown in Fig. 2 expressed as ratios of distances of each band or breakpoint from the centromere to the length of the short arm of each chromosome. Numbers in brackets refer to the number of observations made for each C-band or breakpoint. Positions of C-bands on deletion lines 6RL21, 6RL1807, and 6RL1801 are similar to those on a normal whole 6R chromosome. The higher than expected values for deletion line 6RL22 reflect the occurrence of an interstitial deletion on the short arm of that chromosome.

Chromosome type	C-Band or breakpoint						
	6RS3	6RL13	6RL15	6RL17	6RL21	6RL23	6RL25	Breakpoint
Whole 6R	0.52 ± 0.021 (15)	0.42 ± 0.032 (14)	0.63 ± 0.034 (15)	0.83 ± 0.031 (15)	1.06 ± 0.034 (15)	1.23 ± 0.041 (15)	1.48 ± 0.047 (15)	
Deletion 6RL22	–†	0.50 ± 0.042 (13)	0.82 ± 0.045 (20)	1.17 ± 0.048 (20)	1.48 ± 0.058 (20)			1.54 ± 0.058 (20)
Deletion 6RL21	0.53 ± 0.017 (19)	0.42 ± 0.024 (19)	0.61 ± 0.027 (19)	0.83 ± 0.031 (19)	1.03 ± 0.034 (19)			as for band 6RL21
Deletion 6RL1807	0.54 ± 0.013 (9)	0.41 ± 0.036 (7)	0.60 ± 0.027 (9)	0.83 ± 0.028 (9)				0.99 ± 0.035 (9)
Deletion 6RL1801	0.46 ± 0.018 (14)	0.43 ± 0.00 (2)	0.65 ± 0.036 (14)	0.87 ± 0.034 (14)				as for band 6RL17

† Measurements not taken because of difficulty in recognizing band location.

Table 2. Number of white female nematodes on the roots of wheat sib lines with and without telocentric 6RL and 6RS chromosomes, deletion mutants of rye chromosome 6R and on control susceptible (S) and resistant (R) lines after inoculation with *H. avenae*.

Wheat line	Number plants tested	Average number females per plant
Bioassay 1		
Control lines		
S - cv. Schomburgk	10	16.6 ± 2.6
S - var. Chinese Spring	10	9.5 ± 1.96
R - Substitution 6R(6D)	20	0.050 ± 0.050
Sib lines ± 6R chromatin		
6RL telocentric chromosome		
+ 6RL	7	0.29 ± 0.18
− 6RL	11	16.5 ± 2.17
6R deletion type L22 (backcrossed with cv. Schomburgk)		
+ deletion chromosome	9	4.56 ± 3.82
− deletion chromosome	11	13.6 ± 3.14
6R deletion type L21 (backcrossed with cv. Schomburgk)		
+ deletion chromosome	6	3.17 ± 2.97
− deletion chromosome	13	11.08 ± 2.24
Bioassay 2		
Control Lines		
S - cv. Schomburgk	10	15.2 ± 3.1
S - var. Chinese Spring	10	10.2 ± 2.2
R - Substitution 6R(6D)	8	0.13 ± 0.13
Sib lines ± 6R chromatin		
6RS6RS telocentric chromosome		
+ 6RS	17	12.0 ± 1.6
− 6RS	3	16.3 ± 4.7
6RL telocentric chromosome		
+ 6RL	12	5.17 ± 2.5
− 6RL	8	14 ± 1.2
Bioassay 3		
Control lines		
S - cv. Schomburgk	10	11.9 ± 0.89
S - var. Chinese Spring	10	9.5 ± 1.2
R - Substitution 6R(6D)	10	0.40 ± 0.22
Sib lines ± 6R chromatin		
6R deletion type L1807 (self pollinated)		
+ deletion chromosome	17	0.35 ± 0.19
− deletion chromosome	3	19.7 ± 6.0
6R deletion type L1801 (self pollinated)		
+ deletion chromosome	10	11.2 ± 1.1
− deletion chromosome	10	8.4 ± 1.3

have indicated that progressive loss of DNA occurred from the broken ends of those chromosomes over generations (Beissmann and Mason, 1988) but Werner et al. (1992), Tsujimoto et al. (1999), and Friebe et al. (2001) reported the healing of broken ends of wheat chromosomes by the spontaneous addition of telomeric repeats.

Marker Gene Location

The isozyme PGDR2 was absent in all deletion lines, indicating that the gene *PgdR2* is located on the distal region of the long arm of 6R between the proximal portion of band 6RL22 and the telomere (Fig. 2). The GOT-R2 isozyme was present in three of the deletion lines 6RL22, 6RL21 and 6RL1807 but absent in line 6RL1801 indicating that the gene *Got-R2* is located on the interstitial portion of the long arm in band 6RL18 (Fig. 2). All deletion lines possessed the isozyme α-AMY-R1 indicating that the encoding gene α-*Amy-R1* was located between the centromere and the proximal portion of band 6RL18 of the long arm of 6R (Fig. 2).

Asiedu et al. (1990) reported that the nematode resistance gene was controlled by a single dominant gene on the long arm of chromosome 6R. By testing the resistance reactions of the four deletion lines (Table 2), the site of the resistance gene has been located to the same interstitial chromosome segment as that carrying the gene *Got-R2* (Table 2, Fig. 2). The symbol *CreR* has been assigned to this gene, on the basis of the nomenclature for the nematode resistance gene on chromosome 2B of wheat (Slootmaker et al., 1974).

Future Gene Transfer Strategy

Although no wheat-6R recombinants were found in this study by means of the isozyme markers α-*Amy-R1*, *Got-R2*, and *PgdR2*, the choice of these markers has been substantiated by the subsequent mapping of the *CreR* gene within the same chromosomal region carrying these isozyme loci. The complex structure of the distal region of the 6RL chromosome arm might have reduced the chances of recovery of wheat-rye recombinants between these markers (see earlier). However, the present 6R deletion mutants may provide a means for producing future recombinants and transfer of the CCN gene to a wheat chromosome.

Naranjo and Fernández-Rueda (1991) reported the increased pairing of chromosome 6R with wheat chromosomes, and particularly with group 6 chromosomes of wheat, when 6R telomeric C-heterochromatin was absent. If the nematode resistant deletion lines (6RL22, 6RL21, and 6RL1807) have lost the distal 7R and 3R chromosome segments in addition to the telomeric sequences, the remaining proximal sections of 6R may show sufficient homoeology with group 6 chromosomes of wheat (either entire or with a small terminal deficiency) to enable recombination to occur in a *ph1bph1b* background. It is currently unknown where the exact breakpoints are on 6R which demarcate the translocation breakpoints of the 7R, 3R, and 6R segments. Alonso-Blanco et al. (1994) stated that the 3R and 7R segments on the 6R chromosome of their study represented 25 to 30% of the distal length of the 6RL chromosome arm. Studies are underway to determine this using the current deletion lines.

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