

Table 1. Inheritance of revolute cotyledons (*rc-2*) in the cucumber cross WI 2757 × NCG-093

Generation	No. observed (N:R) ^a	No. expected (N:R)	Expected ratio (N:R)	χ^2	P value
WI 2757 (P ₁)	44:0	44:0	1:0	—	—
NCG-093 (P ₂)	0:16	0:16	0:1	—	—
F ₁	54:0	54:0	1:0	—	—
F ₂	122:41	122:41	3:1	0.002	.96
BC _{1A}	79:0	79:0	1:0	—	—
BC _{1B}	47:33	40:40	1:1	2.45	.11

^a R = revolute; N = normal cotyledons.

young (Figure 1). The size and green color of the mutant cotyledons are the same as the normal cotyledons of WI 2757 (Peterson et al. 1982). The true leaves of the seedlings are also normal, and the cotyledons become difficult to distinguish from normal cotyledons when the plants reach the four true-leaf stage.

In order to study the inheritance of the revolute cotyledons mutant, NCG-093 and WI 2757 were increased by self-pollination and checked for uniformity of cotyledon type to develop parental inbred lines. The two inbreds were crossed using hand-pollination in a greenhouse. The F₁ progeny were self-pollinated to produce the F₂ generation and also backcrossed to each parent to produce the BC_{1A} (F₁ × WI 2757) and BC_{1B} (F₁ × NCG-093).

Seedlings were grown in flats of vermiculite on benches in the greenhouse (temperature 20°C–35°C with a 13–14 h photoperiod). Six days after seeding, plants were evaluated for cotyledon phenotype and classified as revolute or normal.

The cross of normal cotyledons WI 2757 with revolute cotyledons NCG-093 produced all normal F₁ progeny (Table 1). Segregation in the F₂ progeny fit the 3:1 expected ratio ($P > .96$), assuming the trait was controlled by a single recessive gene. Progeny segregation in the BC_{1A} and BC_{1B} generations verified the inheritance pattern for a single recessive gene observed in the F₂ progeny. The BC_{1A} (to NCG-093) segregated in a 1:1 ratio, with an adequate fit to expected values ($P > .05$). No revolute cotyledons seedlings were observed in BC_{1B} (to WI 2757).

We concluded that there was a single recessive gene for revolute cotyledons-2 in NCG-093 for which we propose the symbol *rc-2*. The mutant of Burple Hybrid having revolute cotyledons described by Whelan et al. (1975) was lost, so it was impossible to compare the mutants for similarity or to cross them to test for allelism. Seeds of NCG-093 can be obtained from T.C.W.

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Preferential Male Transmission of an Alien Chromosome in Wheat

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A telocentric chromosome 5H^L from *Elymus trachycaulus* (Link) Gould ex Shinners was transferred into common wheat (*Triticum aestivum* L.). This chromosome was assigned to the homoeologous group 5 of Triticeae species by restriction fragment length polymorphism (RFLP) analysis. Chromosome 5H^L was transmitted in 20% of the female gametes and 97% of the male gametes in the genetic background of wheat, although the expected transmission frequencies of 5H^L through female and male gametes are 25% and 0–5%, respectively. It is likely that a gene located on 5H^L promotes male gamete competition. We suggest that the long arms of homoeologous group 5 chromosomes in Triticeae species carry genes that affect their transmission through male gametes.

Genes from a number of wild species have been successfully transferred into cultivated wheat (Islam and Shepherd 1992;

Jiang et al. 1994a). Production of wheat-alien chromosome addition lines is usually the first step to do such transfers. One common character for most of the wheat-alien monosomic addition lines (42 wheat chromosomes plus one alien chromosome) is the low transmission of the alien chromosome through pollen, often less than 5%. Thus pollen with the additional alien chromosome can hardly compete with normal ones in fertilization. During the past few years we have isolated several wheat-*Elymus trachycaulus* ($2n = 4x = 28$, genomically S'S'H'H') chromosome addition lines (Jiang et al. 1994b; Morris et al. 1990). In the selfed progenies of a monotelosomic 5H^L addition line, the telocentric chromosome 5H^L was detected almost exclusively in all the plants analyzed. This article reports on this rare case of preferential male transmission of 5H^L in the genetic background of wheat.

Materials and Methods

Wheat-*E. trachycaulus* monosomic addition line 5H^L (designated as MA 5H^L, chromosome constitution is $21'' + 5H^L$), monotelosomic addition line 5H^L (MTA 5H^L, $21'' + 5H^L$); 5H^L represents a telocentric chromosome derived from the long arm of 5H^L and monoisosomic addition line 5H^L.5H^L (MIA 5H^L.5H^L, $21'' + 5H^L.5H^L$); 5H^L.5H^L represents an isochromosome derived from the long arm of 5H^L were isolated from the backcrossed progenies of an *E. trachycaulus* × Chinese Spring (CS) wheat hybrid. These lines were used for male and female transmission studies in crosses with CS wheat. Chromosomes 5H^L and 5H^L.5H^L were identified by N-banding analysis (Endo and Gill 1984).

For RFLP analysis, genomic DNA was isolated from young leaf tissue of wheat and the monosomic addition lines. DNA samples were digested with restriction enzyme *Hind*III and blotted to MSI membrane. Prehybridization and hybridization were done at 65°C in $5\times$ SSC, 100 mM NaPO₄ (pH 6.5), 20 mM EDTA, 0.5% SDS, and 0.2 mg/ml denatured salmon sperm DNA. The membranes were washed at 50°C in $0.1\times$ SSC and 0.1% SDS, and exposed to X-ray film. The two group 5-specific RFLP markers, PSR118 and PSR128 (Sharp et al. 1989), were kindly provided by Dr. M. D. Gale, John Innes Center, Norwich, England.

Results

Designation of Chromosome 5H^L

Chromosome 5H^L has two characteristic N-bands near the centromere on the long



Figure 1. N-banding patterns of complete chromosome 5H^t, telocentric chromosome 5H^tL, and isochromosome 5H^tL·5H^tL.

arm (Figure 1). Therefore chromosomes 5H^t, 5H^tL, and 5H^tL·5H^tL can be distinguished from all the wheat chromosomes. RFLP analysis with wheat homoeologous group 5-specific probes indicated that 5H^t belongs to group 5. The RFLP marker, PSR118, assigned to the short arms of group 5 chromosomes, hybridized to a specific DNA fragment derived from the short arm of 5H^t (Figure 2a). Similarly the group 5 long arm marker, PSR128, hybridized to a DNA fragment from the long arm of 5H^t (Figure 2b).

Chromosome Transmission

The telocentric chromosome 5H^tL from a monotelosomic addition line showed male and female transmission frequencies of 0.97 (29/30) and 0.20 (2/10), respectively (Table 1). Based on the previous estimates of transmission of alien chromosomes in the genetic background of wheat (Hyde 1953; Riley 1960), the expected transmission frequencies of 5H^tL through male and female gamete are 0.00–0.05 and 0.25, respectively. The present data indicate a preferential transmission of 5H^tL through the male gametes.

Preferential male transmission was also observed in progeny of selfed MTA 5H^tL. Assuming 0.20 female and 0.97 male transmission of 5H^tL, we expected five ditelosomic addition (21ⁿ + 5H^tLⁿ) and 21 monotelosomic addition plants from a total of 27. This is close to the 6 and 21 plants observed for the respective classes (Table 1). We also analyzed 20 seeds from a selfed population of a double monosomic 5B and 5H^t (20ⁿ + 5Bⁿ + 5H^tⁿ). Nineteen plants had at least one copy of 5H^t. This result indicated that the complete chromosome 5H^t has a similar male transmission behavior as its long arm 5H^tL.

A population of selfed MIA 5H^tL·5H^tL was analyzed for the transmission of the isochromosome 5H^tL·5H^tL. If an alien chromosome has no preferential transmission in the wheat genetic background, 25%

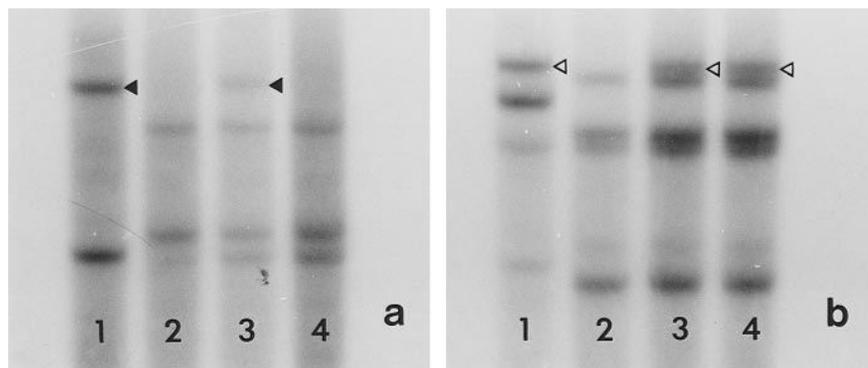


Figure 2. *Hind*III-digested DNA samples from *E. trachycaulus* (lane 1), Chinese Spring wheat (lane 2), monosomic 5H^t addition line (lane 3), and monotelosomic 5H^tL addition line (lane 4) probed with (a) a group 5 short arm marker, PSR118; (b) a group 5 long arm marker, PSR128. Solid arrowheads point to the bands specific to the short arm of 5H^t. Open arrowheads point to the bands specific to the long arm of 5H^t.

monosomic additions and 75% euploid plants will be expected from selfed progenies of a monosomic addition line. While assuming 5H^tL·5H^tL has 20% female and 97% male transmission, similar to 5H^tL, we expected about 20% diisomic addition (21ⁿ + 5H^tL·5H^tLⁿ) and 80% monoisomic addition plants. The observed transmission of 5H^tL·5H^tL was in the middle of these expectations (Table 1). Therefore the isochromosome 5H^tL·5H^tL showed reduced preferential transmission as compared to the telocentric chromosome 5H^tL. About 38% (8/21) of the plants in the selfed progenies of MIA 5H^tL·5H^tL contained a 5H^tL telosome. These telocentric chromosomes may have been derived from misdivision of the isochromosome 5H^tL·5H^tL.

The meiosis of MTA 5H^tL was studied. No abnormal meiotic behavior except lagging 5H^tL was observed at anaphase I and

anaphase II. More than 95% of the pollen grains of MTA 5H^tL were of normal size, with two visible sperm nuclei and one vegetative nucleus.

Discussion

In plants, euploid male gametes generally have a distinct competitive advantage over male gametes with extra chromosomes. The frequency of disomic wheat-alien chromosome additions among the progenies of monosomic additions is usually very low because of the low transmission of the alien chromosome through male gametes. In this study, the transmission of 5H^tL through the female gametes (20%) is within the range (15–45%) of other *E. trachycaulus* chromosomes in the genetic background of wheat. However, 5H^tL was transmitted in 97% of the male gametes, suggesting that the male gametes

Table 1. Transmission of chromosomes 5H^t, 5H^tL, and 5H^tL·5H^tL in the genetic background of wheat

Cross ^a (♀/♂)	Chromosome constitution	No. of plants
CS/21 ⁿ + t'	21 ⁿ + t'	29
	21 ⁿ	1
	Total	30
21 ⁿ + t'/CS	21 ⁿ + t'	2
	21 ⁿ	8
	Total	10
21 ⁿ + t', self	21 ⁿ + t ⁿ	6
	21 ⁿ + t'	21
	21 ⁿ	0
	Total	27
20 ⁿ + 5B ⁿ + 5H ^t ⁿ , self	20 ⁿ + (0–2)5B + (1–2)5H ^t	19 ^b
	20 ⁿ + 5B ⁿ	1
	Total	20
21 ⁿ + i', self	21 ⁿ + i ⁿ	2
	21 ⁿ + i' + t'	2
	21 ⁿ + i'	5
	21 ⁿ + t'	6
	21 ⁿ	6
	Total	21

^a t: 5H^tL; i: 5H^tL·5H^tL.

^b Four plants had a 5H^tL instead of 5H^t.

with 5H^L had a competitive advantage over euploid male gametes. Telocentric chromosome 5H^L was frequently detected in the selfed progenies of MIA 5H^L·5H^L and MA 5H^L (Table 1). Most of these telocentric chromosomes probably originated in the pollen because they can be preferentially transmitted through male gametes. In contrast to 5H^L, the short arm of 5H^S (5H^S) was rarely recovered in the selfed progenies of MA 5H^L. The frequent recovery of 5H^L from the rare misdivision of 5H^L·5H^L and 5H^S again indicates a strong selective advantage of male gametes with 5H^L.

The genetic mechanism of the preferential male transmission of 5H^L is not known, but it seems different from that reported for the gametocidal chromosomes from *Aegilops* species (Endo 1990; Endo and Tsunewaki 1975; Maan 1975; Miller et al. 1982). A gametocidal chromosome will cause abortion of the gametes lacking it, resulting in partial sterility and exclusive transmission of such chromosomes through both male and female gametes (Endo 1990). In the present case, more than 95% of the pollen from MTA 5H^L appeared normal, and both MA 5H^L and MTA 5H^L are fully fertile. In addition, no chromosomal structural changes were observed in the progenies derived from MA 5H^L, MTA 5H^L, and MIA 5H^L·5H^L, while chromosomal mutations were frequently detected in the progenies of wheat plants containing gametocidal chromosomes (Endo 1990). Another distinct characteristic of 5H^L is its preferential transmission only through male gametes. Gametocidal chromosomes are transmitted preferentially through both male and female gametes (Endo 1990).

A possible genetic mechanism is that a gene located on 5H^L may be responsible for its preferential male transmission. This gene makes male gametes more competitive than the gametes lacking it. Supporting evidence for this hypothesis is that another homoeologous group 5 chromosome, 5S^t, from *E. trachycaulus* may have a similar gene that enhances male gamete transmission. Although this chromosome is not preferentially transmitted through male gametes like 5H^L, it was detected more frequently in the backcrossed derivatives of the *E. trachycaulus* × CS hybrids than the other six S^t genome chromosomes.

The long arms of wheat chromosomes 5A, 5B, and 5D may also carry important genes for transmission of male gametes. Ditelosomic 5AS, 5BS, and 5DS, in which

the long arm is absent, are among the few unavailable ditelosomic lines of CS wheat (Sears and Sears 1978). It has been impossible to recover ditelosomic 5AS, 5BS, and 5DS plants from progenies of selfed plants with the chromosome constitution of 20' + 5AS' + 5AL', 20' + 5BS' + 5BL', and 20' + 5DS' + 5DL', respectively. It is most likely that male gametes of 20' + 5AS', 20' + 5BS', and 20' + 5DS' are not functional or they cannot compete with those of 20' + 5AS' + 5AL', 20' + 5BS' + 5BL', and 20' + 5DS' + 5DL', respectively.

In the progenies of heterozygous 5A (or 5B or 5D) deletion lines, homozygous deletion plants were never recovered if the deleted 5A (or 5B or 5D) had less than 50% of the long arm (Endo and Gill 1996). This result suggests that male gametes with deleted 5A (or 5B or 5D) are not functional or are unable to compete with those having normal 5A (or 5B or 5D). The genes that are critical for male gamete transmission are possibly located on the proximal half of chromosomes 5A, 5B, and 5D.

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The Journal of Heredity 1998:89(1)

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Low Allozyme Diversity in *Schwalbea americana* (Scrophulariaceae), an Endangered Plant Species

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Schwalbea americana, a hemiparasitic member of the Scrophulariaceae, is an early successional, fire-dependent species of the eastern coastal plain of North America. We sampled 13 populations across the range of this endangered perennial herb to describe allozyme diversity at 15 presumptive loci. Genetic diversity was low for the species, within populations, and for polymorphic loci ($H_{es} = 0.006$; $H_{ep} = 0.005$; $H_t = 0.028$). Three of the 15 loci (20%) were polymorphic across the species' range, but frequencies of uncommon alleles were uniformly low (mean $P = .05$). No polymorphism was detected in seven populations. Population extinctions and decreases in population sizes, coupled with habitat fragmentation, may account for the low genetic diversity. The fugitive life-history characteristics of this shade-intolerant species presumably have also contributed to the loss of genetic diversity by predisposing the species to founder effects and population extinctions.

Schwalbea americana comprises a monotypic genus in the figwort family, Scrophulariaceae. Like many members of this family, *S. americana* is a hemiparasite, ob-