



Cause of tall off-types and estimation of genome relationships in wheat
by Eric William Storlie

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
Crop and Soil Science

Montana State University

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Abstract:

Wheat breeders work to develop genetically uniform wheat (*Triticum aestivum* L.) cultivars. Phenotypic off-types in a population may be an indication of residual heterozygosity. Tall off-types occur in some spring wheat varieties at a frequency as high as 0.20%. Cytological analysis of offspring from selfed tall off-types showed a high proportion of monosomic chromosome numbers, indicating that aneuploidy may be associated with the tall off-types. The offspring of tall off-type selections were planted and analyzed for height-segregation and production of nullisomic plants. Nullisomic 4B and 4D conditions were determined by PCR (polymerase chain reaction) using primer set G10 which amplifies distinct DNA fragments for chromosomes 4A, 4B and 4D. Nullisomic 4B and 4D offspring were identified by the absence of a DNA fragment from respective chromosomes. The offspring analysis for segregation and production of nullisomics was used to infer the chromosomal constitution of the tall off-type selection. Results of this study indicate that semidwarf wheat with height-reducing genes, *Rht1* or *Rht2*, produces tall off-types when monosomic 4B or 4D conditions occur, respectively. *Rht1* resides on chromosome 4B and *Rht2* on chromosome 4D. Six varieties with *Rht1* genotypes produced an average frequency of 0.0016 monosomic 4B wheat, and 5 varieties with *Rht2* genotypes produced an average frequency of 0.0005 monosomic 4D wheat. Variation for occurrence and effect of monosomic 4B and 4D conditions between *Rht1* and *Rht2* may be utilized in a breeding program to minimize the problem of tall off-types.

In a second set of experiments, amplified DNA fragments from chromosomes 4A, 4B and 4D were used for sequence comparisons to estimate genome relationships. The DNA fragments were cloned and sequenced. Aligned sequences were compared by Parsimony and Distance matrix methods.

Parsimony analysis produced a tree that grouped, with 93% confidence, the 4A and 4D genomes on a separate clade from the B genome. Distance matrix grouped 4A and 4D genomes with 61% confidence. Results indicate the A and D genomes are more closely related than either are to the B genome.

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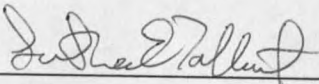
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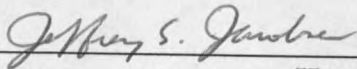
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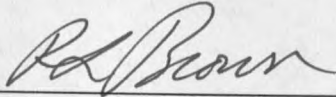
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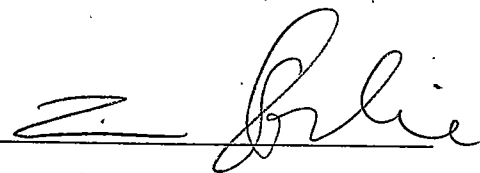
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ABSTRACT

Wheat breeders work to develop genetically uniform wheat (*Triticum aestivum* L.) cultivars. Phenotypic off-types in a population may be an indication of residual heterozygosity. Tall off-types occur in some spring wheat varieties at a frequency as high as 0.20%. Cytological analysis of offspring from selfed tall off-types showed a high proportion of monosomic chromosome numbers, indicating that aneuploidy may be associated with the tall off-types. The offspring of tall off-type selections were planted and analyzed for height-segregation and production of nullisomic plants. Nullisomic 4B and 4D conditions were determined by PCR (polymerase chain reaction) using primer set G10 which amplifies distinct DNA fragments for chromosomes 4A, 4B and 4D. Nullisomic 4B and 4D offspring were identified by the absence of a DNA fragment from respective chromosomes. The offspring analysis for segregation and production of nullisomics was used to infer the chromosomal constitution of the tall off-type selection. Results of this study indicate that semidwarf wheat with height-reducing genes, *Rht1* or *Rht2*, produces tall off-types when monosomic 4B or 4D conditions occur, respectively. *Rht1* resides on chromosome 4B and *Rht2* on chromosome 4D. Six varieties with *Rht1* genotypes produced an average frequency of 0.0016 monosomic 4B wheat, and 5 varieties with *Rht2* genotypes produced an average frequency of 0.0005 monosomic 4D wheat. Variation for occurrence and effect of monosomic 4B and 4D conditions between *Rht1* and *Rht2* may be utilized in a breeding program to minimize the problem of tall off-types.

In a second set of experiments, amplified DNA fragments from chromosomes 4A, 4B and 4D were used for sequence comparisons to estimate genome relationships. The DNA fragments were cloned and sequenced. Aligned sequences were compared by Parsimony and Distance matrix methods. Parsimony analysis produced a tree that grouped, with 93% confidence, the 4A and 4D genomes on a separate clade from the B genome. Distance matrix grouped 4A and 4D genomes with 61% confidence. Results indicate the A and D genomes are more closely related than either are to the B genome.

ONE

INTRODUCTION

This dissertation describes three research projects which are separated into chapters two, three and four. Each project follows a progression of related ideas and hypotheses. The initial idea was provoked by an occurrence of tall off-types in a recently released Montana wheat variety, 'Hi-Line'. The persistent frequency of about 0.20% tall off-types was considered problematic and a curiosity because the frequency could not be controlled by culling the off-types. A research project was designed to explore the question of tall off-types, and subsequent projects were designed to explore questions spawned by the initial results.

Chapter two describes the analysis of tall off-types in Hi-Line. The possibility of impure genotypes was discounted by comparing glutenin banding patterns of the off-types with the Hi-Line banding pattern. A cytological analysis of the off-type offspring indicated that some of the offspring were monosomic and nullisomic. These conditions suggested an aneuploid chromosomal constitution for the tall off-types.

The next step involved identification of the chromosomes involved with the aneuploid condition. Cytological techniques such as C-banding allow the

identification of chromosomes, but these techniques are difficult and time consuming. Another approach involves the use of molecular DNA markers that are chromosome specific. The presence and absence of DNA marker bands indicate the presence and absence of chromosomes. The molecular approach can be used to identify absent chromosomes in nullisomic wheat. As indicated by the cytological analysis, some of the offspring were nullisomic. A missing chromosome 4B was identified by PCR (Polymerase Chain Reaction) using primer set G10, which amplified distinct DNA fragments for chromosomes 4A, 4B and 4D. Tall off-types that produced height-segregating and nullisomic 4B offspring were inferred to have a monosomic 4B condition.

Chapter two concludes that the monosomic condition of chromosomes 4B and 4D, containing the *Rht1* (*Rht1Rht1rht2rht2*) and *Rht2* (*rht1rht1Rht2Rht2*) genotypes, respectively, may cause tall plants. An effective methodology that relied on an offspring analysis was developed to determine a cause and effect for tall off-types in Hi-Line.

Chapter three approaches a follow-up question on variation for the occurrence and effect of tall off-types. Eleven semidwarf wheat varieties with either *Rht1* or *Rht2* genotypes were compared for frequencies and height effects of tall off-types. The same basic methodology developed in chapter two was used for the genotypic comparisons. Results

in chapter three indicate that 5 varieties with *Rht1* and 3 with *Rht2* genotypes had an occurrence of tall off-types caused by monosomic 4B and 4D conditions, respectively. Three varieties included in the analysis produced no detectable monosomics, which may be due to relatively small population sizes (*n*) in two of the populations. Comparisons indicate that *Rht2* genotypes have a lower frequency of tall off-types than varieties with *Rht1* genotypes. Comparisons within *Rht1* or *Rht2* genotypes indicate there were no genotypic influences on the occurrence of tall monosomic wheat. Comparisons for effect on height indicate significant differences between *Rht1* and *Rht2* genotypes and significant differences within the *Rht1* genotypes. This chapter concludes that *Rht1* and *Rht2* genotypes of semidwarf wheat may produce tall monosomic plants; variation was detected for monosomic conditions and for height effects between *Rht1* and *Rht2* genotypes and for height effects within *Rht1* genotypes. This variation may be utilized to minimize the occurrence and effect of tall off-types during a breeding procedure.

Chapters two and three relate to questions of tall off-types and rely on a common methodology which utilizes PCR to amplify DNA sequences from each of the 3 wheat genomes. DNA bands were associated with chromosomes 4A, 4B and 4D using tetrasomic 4 (nullisomic 4) stocks. PCR amplification of euploid wheat appears as three bands on an electrophoresis

gel. Amplification of nullisomic 4A, 4B or 4D wheat appears as 2 bands that indicate the nullisomic condition. Thus the three DNA sequences were clearly amplified from each of the genomes.

A question of evolutionary relationships between the A, B and D genomes of wheat was spawned by the identification of homologous DNA fragments amplified by PCR. Chapter 4 approaches the question of genome relationships. Each of the DNA fragments associated with chromosomes 4A, 4B and 4D were cloned and sequenced. The nucleotide sequences were aligned and compared using phylogenetic analyses. Results indicate that the A and D genomes are more closely related to each other than either are to the B genome. These results correspond with several chromosome pairing studies and provide nucleotide sequence data for more evidence on evolutionary relationships in wheat.

The three chapters approach questions related to wheat genetics and breeding. Chapter two shows that a frequency of tall off-types in a wheat variety, Hi-Line, is caused by a monosomic 4B condition. This result suggests that tall off-types may be an inherent and unavoidable feature of semidwarf wheat. Chapter 3 shows that both *Rht1* and *Rht2* semidwarf wheat may produce tall off-types and that there is some variation for occurrence and height effects between *Rht1* and *Rht2* genotypes and for height effects within *Rht1* genotypes. This variation may be used in a breeding program

to minimize the problem of tall off-types. Chapter 4 shows that DNA sequence comparisons in wheat may be an effective approach to evolutionary questions. It also bolsters evidence that the A and D genomes are more closely related. An understanding of evolutionary relationships may be important for wheat breeders to utilize ancestral germplasm for intraspecific crosses.

TWO

CAUSE OF TALL OFF-TYPES IN A
SEMIDWARF SPRING WHEATLiterature Review

Wheat cultivars are released by breeders as homozygous inbred genotypes that are genetically and phenotypically uniform. Phenotypic variation is caused by outcrossing, mechanical mixtures and aneuploid conditions (Hollingshead, 1932; Love, 1943; Myers, 1938; Powers, 1932; Riley and Kimber, 1961; Thompson and Robertson, 1930). Products of outcrossing and mechanical mixtures are easily culled, if phenotypically distinct. Occurrences of aneuploids may be difficult to manipulate because of a random and spontaneous nature.

Wheat is relatively tolerant of some aneuploid conditions because of its hexaploid ($2n=6x=42$) condition. Sears (1954) documented tetrasomic, trisomic, monosomic and nullisomic conditions for most of the chromosomes of wheat, and he discovered that most of these aneuploid conditions were viable. Several early studies identified an occurrence of unpaired chromosomes at metaphase I in different varieties of wheat (Hollingshead, 1932; Myers, 1938; Powers, 1932; Riley and Kimber, 1961; Thompson and Robertson, 1930). Riley and Kimber (1961) observed pollen cells of five

hexaploid wheat varieties and estimated a frequency of 0.0028 pairing failure per bivalent. The same frequency of pairing failure was estimated for five diploid ancestors of hexaploid wheat. Riley and Kimber (1961) suggest these chromosomal anomalies will produce a frequency of 0.00186 monosomic wheat from euploids, and in the diploid ancestors, the anomalies will not produce sporophytes.

Monosomic wheat was estimated by Sears (1953) to produce frequencies of 0.73 monosomics, 0.24 euploids and 0.03 nullisomic offspring. These proportions were calculated from an estimate of chromosomal constitutions of female and male gametes and respective transmissions to offspring. Seventy-five percent of female gametes are expected to have 20 chromosomes because univalents should lag and frequently become lost from the nucleus. Ninety-six percent of male gametes are expected to have 21 chromosomes because pollen with 21 chromosomes should outcompete those with 20 chromosomes for successful unions with eggs (Morrison, 1953; Sears, 1953).

Sears (1954) analyzed the offspring of monosomic Chinese Spring wheat for each chromosome and estimated frequencies of nullisomic offspring. Frequencies ranged from 0.9 percent for chromosomes 5B and 5D and 7.6 percent for chromosome 3A. Chromosomes 4B and 4D had frequencies of 6.4 and 5.9 percent, respectively (chromosomes 4B and 4D are relevant to this study). A small proportion of monosomic

offspring are isochromosomal or telocentric due to misdivision at meiosis (Love, 1943; Morrison, 1953; Sears, 1952). Sears (1954) estimated frequencies of telocentric and isochromosomal offspring produced from monosomic plants for each chromosome. Monosomic 4B and 4D wheat produced 8.4 and 0.9 percent telocentric or isochromosomal 4B and 4D offspring, respectively. The offspring of monotelocentric and monoisochromosomal plants produced offspring that were nullisomic, monotelocentric or monoisochromosomal, ditelocentric or diisochromosomal, or telocentric and isochromosomal (Sears, 1954).

Aneuploidy in wheat may result in an occurrence of phenotypic offtypes in wheat varieties (Love, 1943; Riley and Kimber, 1961; Worland and Law, 1985). Variation between varieties for the occurrence of aneuploid conditions suggests meiotic instability is heritable (Myers, 1938; Powers, 1932; Riley and Kimber, 1961). Riley and Kimber (1961) further suggest the phenotypic effect of certain aneuploid conditions varies between varieties.

Worland and Law (1985) showed tall offtypes that occurred in a cultivar of spring wheat, 'Brigand', were due to a monosomic 4D condition. They estimated monosomic 4D plants occurred with a frequency of 0.13% in a particular population that had been culled of off-types in the previous generation. Monosomic conditions were associated with tall offtypes from three other varieties - 'Hobbit', 'Frontier'

and 'Guardian' - though frequencies of their occurrences were not determined. Worland and Law (1985) noted that there were significant height differences between monosomic plants of Hobbit and the other three varieties.

Chromosome 4B contains a gene, *Rht1* that confers gibberellin insensitivity and is involved with height reduction in some semidwarf and dwarf wheats (Gale et al., 1975). Another gene, *Rht2*, on chromosome 4D, serves a similar function (Gale and Marshall, 1976).

A recently released semidwarf cultivar from Montana, Hi-Line, has a frequency of 0.20% tall off-types that may be 17 to 34% taller than the population mean (Lanning et al., 1992). The objective of this investigation was to determine the genetic basis for tall off-types in Hi-Line and to determine segregation frequencies of off-type offspring, using cytological and molecular techniques and field observations for analysis.

Materials and Methods

Observations of Tall Off-Types in Hi-Line

A traditional head-row/line-row cultivar purification procedure was followed prior to the release of Hi-Line (Lanning et al., 1992). In 1988, 550 lines consisting of approximately 50 individuals per line, derived from single heads of MT8402 - subsequently named Hi-Line - were evaluated for phenotypic uniformity in Bozeman. Due to lack

of uniformity, including the presence of tall off-types, 102 lines were discarded. The remaining 448 lines were evaluated as line rows in 1989, and 208 were discarded largely due to the presence of tall off-types. Of approximately 84,000 individuals evaluated in this nursery, 199 (0.2%) were classified as tall. Seed harvested from the remaining 240 line rows were planted in 1990, and 110 rows were eliminated due to the presence of tall off-types (with an average of 22% taller than the population mean). Seed from the remaining 130 lines were bulked to form breeder seed of Hi-Line.

Segregation Analysis of *Rht1* and *Rht1* Crosses

Euploid Hi-Line was reciprocally crossed with six varieties containing known height-reducing genes. 'Siete Cerros', 'Inia' and 'Nacozari' are homozygous for *Rht1*; 'Ciano', 'Pavon' and 'Jaral' are homozygous for *Rht2* (Kihara, 1983).

Glutenin Analysis

Seeds were harvested from tall off-types and a seed from each plant was evaluated for its glutenin banding pattern using SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) to determine the genotypic purity of Hi-Line off-types.

Seed storage proteins were extracted by method of Laemmli (1970). Each seed was ground with a mortar and

pestle and suspended in 500 μ L of 70% ethanol (C_2H_5OH). The samples were briefly vortexed and incubated in a 55 °C waterbath for 1 h and centrifuged for 2 min at 8,000 X g. A 250 μ L aliquot of supernatant was removed from each sample and placed in a 4 °C refrigerator for 1 h. The cold supernatants were dried in a vacuum centrifuge (Savant, Farmingdale, NY). The pellets were resuspended in a cracking buffer - 23% glycerol, 80 mM sodium dodecyl sulfate, 145 mM Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol, 1.7 mM bromo phenol blue (3',3'',5',5''-tetrabromophenolsulfonephthalein), 2.1 mM xylene cyanole FF and 30 mM β -mercaptoethanol.

Glutenins were separated through a 12% polyacrylamide gel - 12% acrylamide 460 mM Tris, 0.10% SDS, 0.01% Ammonium Persulfate, $(NH_4)_2S_2O_8$, 5 mM Temed, N,N,N',N'-tetramethylethylenediamine - using 80 volts for 16 h. Gels were stained for two h in 1.2 mM Coomassie Blue. The analysis for genetic purity involved a comparison of glutenin banding patterns of each tall off-type sample with a euploid Hi-Line sample.

Cytological Analysis

Ten randomly selected seeds from off-type Hi-line were germinated in petri dishes on water-soaked, 3mm filter paper (Whatman, Maidstone, England). Root-tip squashes followed the smear technique of Tsuchiya (1971). After 2 d, when

roots were at least 1 cm in length, the root tips were placed in H₂O at 4 °C for 24 h, then fixed in 45% glacial acetic acid (CH₃CO₂H) for 0.5 h. After fixing, root tips were placed in 4 °C H₂O prior to squashing and staining in acetocarmine solution (2% carmine and 45% glacial acetic acid) for cytological analysis.

DNA Extraction

Approximately 10 mg of leaf tissue was removed from wheat seedlings for DNA extraction by method of Lassner et al. (1989). Each tissue sample was ground with a glass rod in 1.5 mL microcentrifuge tubes (National Sci., San Rafael, CA) containing 100 µL extraction buffer - 0.77 mM sorbitol, C₆H₁₄O₆, 1.8 mM Tris, 0.06 mM EDTA, 13.7 mM NaCl₂, 0.06 mM CTAB (Hexadecyltrimethyl-ammonium Bromide), C₁₆H₃₃N[CH₃]Br, 0.12 mM Sarkosyl (N-Lauroylsarcosine), C₁₅H₂₈NO₃Na, 1.82 mM β-mercaptoethanol - and 100 mg sand, and the solution was heated to 65 °C for 30 min. Samples were centrifuged for 5 min, and the supernatants were added to 60 µL isopropanol, mixed, and chilled at -20 °C for 30 min. Samples were centrifuged for 5 min to form a pellet, and the supernatants were discarded. Pellets were washed with 60 µL 70% ethanol and the previous step was repeated. The pellets were resuspended in 40 µL TE (10mM Tris, 1 mM EDTA). These DNAs were used in PCR reactions.

Polymerase Chain Reaction Primers

Two RFLP clones for chromosome 4 were obtained from B.S. Gill, Kansas State Univ. (Gill et al., 1991). These clones, labeled G10 and D21, were sequenced at both ends by the dideoxy chain termination method (Sanger et al., 1977). Primers of 20 bases in length were designed from the sequenced clones and were synthesized with a PCR-mate 391 DNA synthesizer (Applied Biosystems Inc., Foster City, CA), using standard phosphoramidate chemistry. Primer sets WG464 and ST4-6, specific for loci on barley chromosome 4, were obtained from T. Blake (Montana State Univ., Bozeman). Primer concentrations were adjusted to 100 ng/ μ L with a spectrophotometer (Varian Techtron, Australia). The sequences of the primers (5'-3') are:

G10L-GTGTTGATGTCCTTGAGGCC, G10R-TGTCCAGCTTCAGCGAGTAC,
D21L-TCTTCCAGTTAGAGATCTCC, D21R-TCGTTCGTACTAGTAGTACC,
WG464L-AGGACTGTGAAGATGCTACT, WG464R-AGTCCAAATGATGTCACAGG,
ST4-ATCCACAGCGGCTGTTCCAC, ST6-CTTGGCCACCGTCATGGTCT.

Primer Evaluation

The three sets of primers were tested for PCR amplification on Chinese Spring wheat, tetrasomic-4D (nullisomic-4A), tetrasomic-4B (nullisomic-4D) and progeny of tetrasomic-4D (monosomic-4B) obtained from USDA-ARS (Columbia, MO). The progeny of monosomic Chinese Spring are expected to include a high proportion of nullisomic-

tetrasomic individuals (E.R. Sears, 1989, personal communication). Also included was *T. speltoides* (Tausch) Gren. ex Richter, a B genome relative of *T. aestivum*.

Polymerase Chain Reaction Conditions

Twenty-five μL reaction mixtures for PCR contained 2.5 μL 10x buffer (500 mM KCl, 100 mM Tris and 1% Triton X-100), 50 μM each of dATP, dCTP, dGTP and dTTP, 1.5 mM MgCl_2 , 400 nM of each primer, 0.6 unit *Taq* polymerase, and 100 ng of genomic DNA. Approximately 60 μL of light mineral oil overlaid each reaction mixture. Reactions occurred in 0.5 mL microfuge tubes (West Coast Scientific, Inc., Hayward, CA) that were thermocycled in a model 50 Tempcycler (Coy Laboratory Products Inc., Grass Lake, MI) with the following temperature conditions: 94 °C for 4 min followed by 30 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1.2 min, and ending with 72 °C for 7 min.

Polymerase Chain Reaction Product Analysis

The reaction products were digested with 1.7 units of *Hinf*I or *Rsa*I (New England Biolabs, Beverly, MA) per reaction mixture for 1 h at 37 °C. PCR products were electrophoresed through a 7% polyacrylamide gel - 7% acrylamide, 1x TBE (44 mM Tris, 44 mM boric acid and 1.0 mM EDTA), 4 mM ammonium persulfate, 3.3 mM temed - immersed in 0.5x TBE buffer. Gels were stained with ethidium bromide and photographed over ultraviolet light.

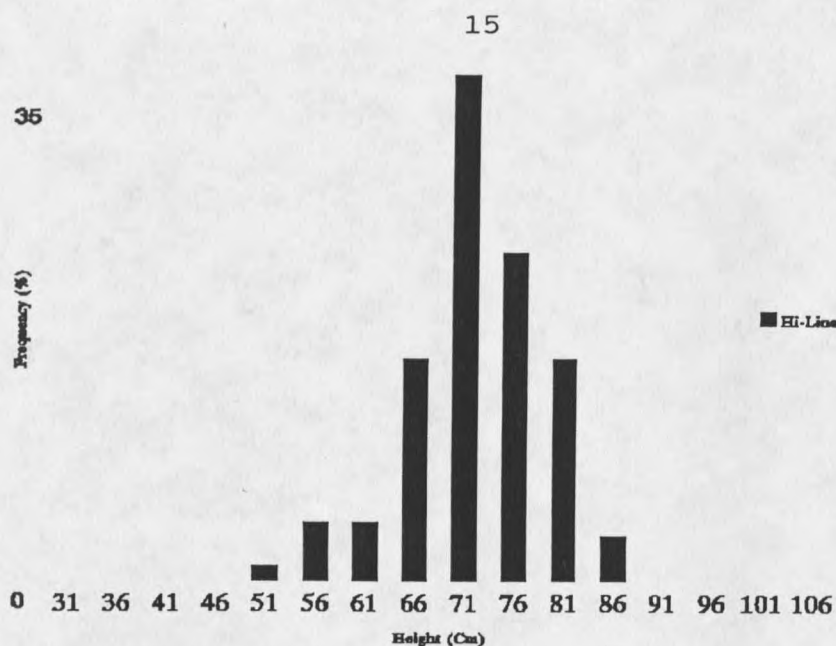


Figure 1. Height distribution of euploid Hi-Line wheat, in 1991.

Tall Off-Type Selection and Analysis

Fifty tall off-type plants were harvested from a line-row plot of Hi-Line with an estimated population size of 28,248 plants. Off-type selection was based on the height-distribution of euploid Hi-Line (Figure 1), and plants with heights taller than the euploid height-range were harvested in 1991 for further analysis. Seeds from 10 off-type Hi-Line plants were planted in pots, and 138 Gen₁ (first generation) offspring were grown to maturity in 1991 under greenhouse conditions. Second generation lines were planted in individual rows in the field in 1992 and evaluated for morphological and molecular (PCR) variation. Offspring produced from monosomic 4B Hi-Line had a height-distribution which is graphically compared with a euploid distribution in

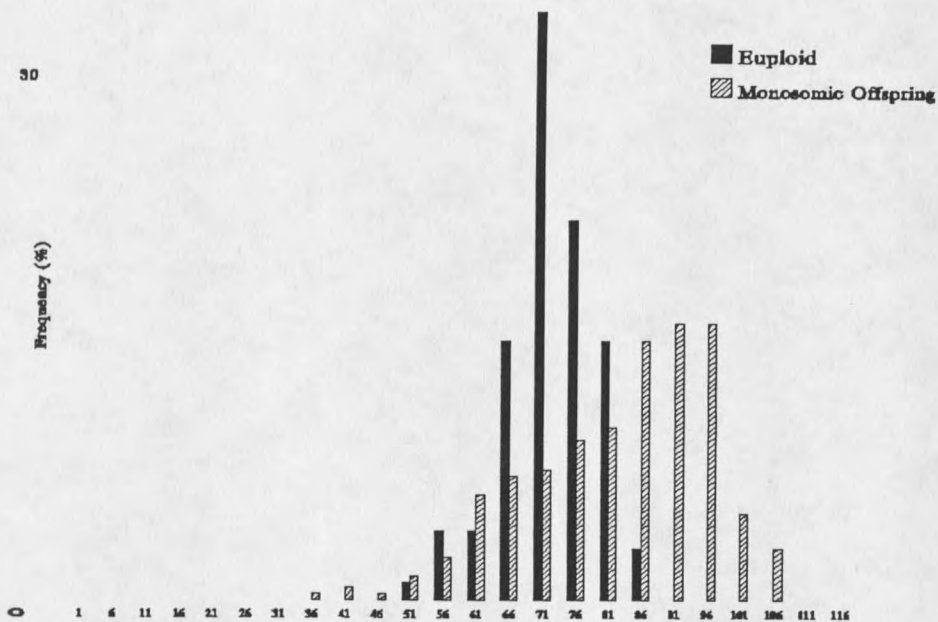


Figure 2. Height distributions of uniform euploid and segregating monosomic offspring of Hi-Line wheat, in 1994.

Figure 2. Fifty rows segregating for height, with a total 1215 plants, were selected for an evaluation of fertility and morphology. The DNA extracts from 68 randomly selected Gen_1 and 113 randomly selected Gen_2 plants were amplified for PCR analysis, using the G10 primer, when plants were at a two-leaf stage. The DNA from a subset of plants was also amplified using WG464 and ST4-6 to confirm results with G10. Plants showing a nullisomic 4B banding pattern were monitored through maturity to distinguish the nullisomic 4B morphology. Plants from Gen_1 were classified as monosomic 4B if the Gen_2 family segregated for height and as disomic 4B if the Gen_2 family was height-uniform. Plants from Gen_2 were classified as monosomic 4B if the height measured more

than 17% above the mean. Plants were classified as nullisomic 4B if sterile and with a nullisomic 4B morphology.

Results and Discussion

Segregation Analysis

The F_2 populations of Hi-Line/*Rht1Rht1rht2rht2* genotypes did not segregate for plant height. The height distribution of Hi-Line/Siete Cerros illustrates a nonsegregating population (Figure 3). The F_2 populations of Hi-Line/*rht1rht1Rht2Rht2* genotypes segregated for plant height. The height distribution of Hi-Line/Pavon illustrates a height segregating population (Figure 3).

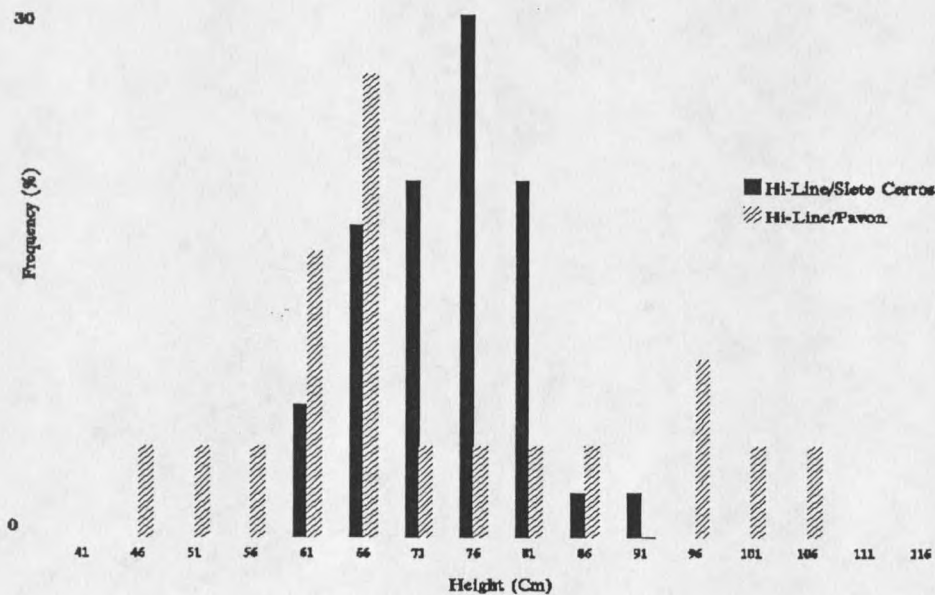


Figure 3. Height distributions of F_2 of Hi-Line/Siete Cerros (genotype - *Rht1Rht1rht2rht2*) and Hi-Line/Pavon (genotype - *rht1rht1Rht2Rht2*).

This result indicates Hi-Line contains *Rht1*, which is on chromosome 4B.

Protein and Cytological Analysis

The glutenin banding patterns of a sample of tall off-type offspring were identical with Hi-Line indicating varietal purity and no outcrossing. An example of uniform banding patterns is shown in Figure 13 (Appendix), lanes c-g. The cytology of 10 offspring of tall off-types revealed 1 disomic: 8 monosomic: 1 nullisomic. This segregation implicated an aneuploid condition with the tall off-types. The sample number evaluated cytologically was too small for a proper estimate of frequency information and did not allow identification of the monosomic chromosome. However, the disproportionately high number of monosomics indicate the parent plant was monosomic (Morrison, 1953; Person, 1956; Sears, 1953, 1954). This corresponds with the results of Worland and Law (1985) that suggest a monosomic 4D condition for tall off-types in Brigand wheat. Subsequent molecular and morphological analyses of segregating progeny were designed to confirm the putative monosomic condition, to determine the monosomic chromosome and to determine the likely progression of Hi-Line tall off-type frequencies in segregating generations.

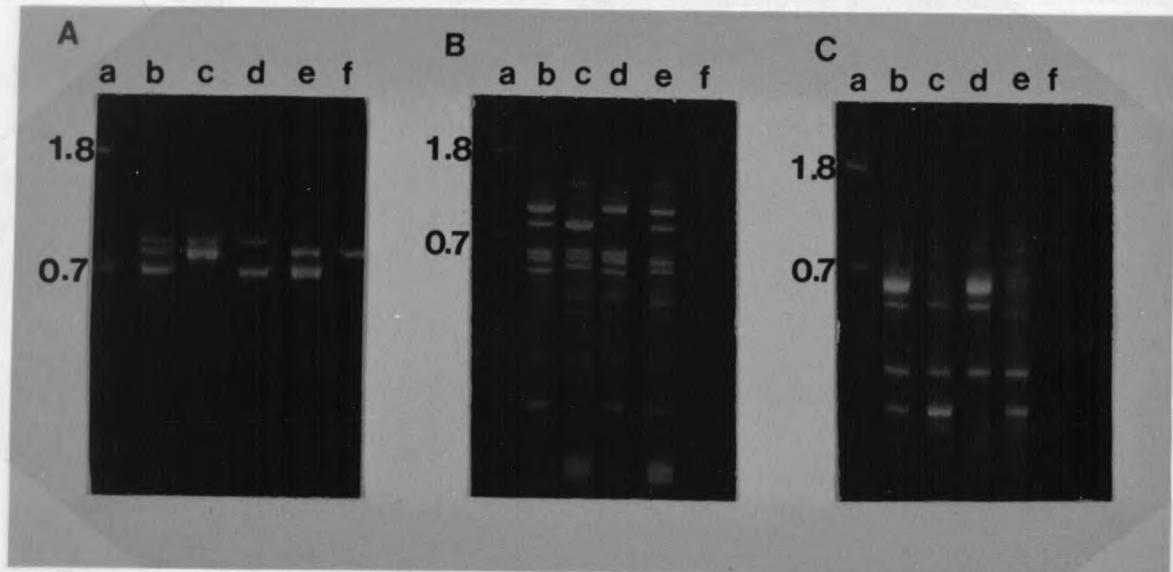


Figure 4. PCR products of three primer sets used to amplify sequences on euploid and aneuploid wheat. Panel A: primer set G10 products digested with *HinfI*; Panel B: primer set WG464 products digested with *RsaI*; Panel C: primer set ST4-6 products digested with *HinfI*. Lanes a, pUC19 digested with *RsaI* (1.8 and 0.7 Kb fragments); b, euploid Hi-Line; c, nullisomic 4D CS (Chinese Spring); d, nullisomic 4B CS; e, nullisomic 4A CS; F, euploid *Triticum speltoides*.

Primer Evaluation

Primer set G10 products, digested with *HinfI*, were polymorphic for the aneuploid wheat DNA (Figure 4). Three distinct fragments - 860 bp, 760 bp, and 650 bp - were produced from disomic DNA samples (lane b). One of these bands was absent for each of the nullisomic group 4 wheat DNA: the 650 bp fragment was absent in nullisomic 4D (lane c), the 760 bp fragment was absent in nullisomic 4B (lane

d), and the 860 bp band was absent in nullisomic 4A (lane e). The DNA of *T. speltoides* produced one fragment of 760 bp (lane f). These results indicate that the 650 bp fragment derives from chromosome 4D, the 860 bp fragment from chromosome 4A, and the 760 bp fragment from chromosome 4B.

Primer set WG464 products, digested with *RsaI*, were polymorphic for disomic and nullisomic 4B and 4D wheat DNA (Figure 4B). Two fragments - 860 bp and 760 bp - were of interest out of several fragments produced from disomic wheat DNA (lane b). The 860 bp fragment was absent from nullisomic 4D wheat (lane c) and the 760 bp fragment was absent from nullisomic 4B wheat (lane d). These results indicate that the 760 bp fragment derives from chromosome 4B and the 860 bp fragment from chromosome 4D. Primer set ST4-6 products, digested with *HinfI*, were polymorphic for disomic and aneuploid wheat (Figure 4C). Four fragments - 490 bp, 465 bp, 370 bp and 350 bp - were produced from disomic wheat (lane b). The 350 bp fragment was absent in nullisomic 4B (lane d). This indicates that the 350 bp fragment derives from chromosome 4B. The polymorphic banding patterns of nullisomic 4D and 4A wheat (lanes c and e, respectively) are confusing and considered poor markers for the respective chromosomes. Primers WG464 and ST4-6 were used to check the consistency of information provided by primer G10. Primer D21 was specific for chromosome 4D,

under our conditions, and did not amplify sequences from the other genomes (data not shown).

Analysis of Tall Off-Type Offspring

The DNA from sixty-eight Gen₁ offspring, grown under greenhouse conditions, and 113 Gen₂ offspring, grown under field conditions, were amplified with the G10 primer set. Twenty per 68 Gen₁ plants and 20/113 Gen₂ plants revealed nullisomic 4B banding patterns. These plants were monitored through maturity and were self-sterile, as described by Sears (1954). The PCR banding patterns for disomic, monosomic and nullisomic 4B plants are shown in Figure 5. Five nullisomic 4B plants were fertilized with pollen from disomic plants and subsequently produced seed. This suggests that the nullisomic 4B condition results in male sterile plants. Other morphological characteristics of nullisomic 4B wheat were: an average height of 26% below the mean disomic height, an average tiller count of 80% fewer tillers than the mean disomic count, and an average flowering date of 10 d later than the mean disomic flowering date.

Polymerase chain reaction amplification of tall off-type offspring indicates that 22% of the offspring was nullisomic 4B, which implicates a monosomic 4B condition of the tall off-type parents (Morrison, 1953; Sears, 1953; 1954). To test the fidelity of primer set G10, 30 offspring

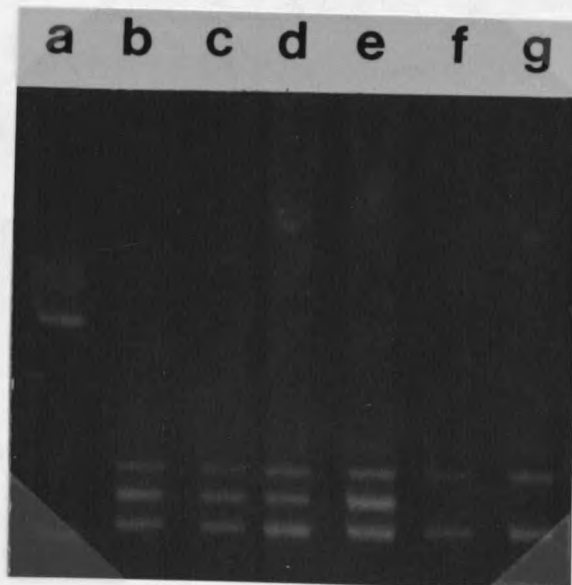


Figure 5. The DNA sequences of euploid and aneuploid Hi-Line wheat were amplified by PCR with primer set G10 and digested with *Hinf*I. Lane a, pUC19 digested with *Rsa*I; b and c, euploid Hi-Line; d and e, monosomic 4B Hi-Line; f and g, nullisomic 4B Hi-Line, which are missing 760 bp DNA fragments.

of average-height Hi-Line, assumed to be disomic, were analyzed by PCR using the G10 primer set. All samples generated disomic banding patterns. To test the reproducibility of primer set G10 with alternate primer sets WG464 and ST4-6, a subset of 15 disomic 4B and 15 nullisomic 4B DNA samples, as identified by G10, were amplified with each of the primer sets. Each primer set generated 15 disomic 4B and 15 nullisomic 4B banding patterns for corresponding samples.

One aberrant Gen_2 line was observed. In a row containing 13 plants, each plant showed a nullisomic 4B banding pattern, using primer set G10 and ST4-6, though only four were self-sterile and had the nullisomic 4B morphology. The other nine had heights ranging from normal to tall, had average flowering dates and had average tiller counts. When these plants were analyzed using primer WG464, four amplifications showed the disomic banding pattern. One explanation for the incongruity may be the occurrence of telocentrics and isochromosomes as described by Sears (1954), though this aberration will require further analysis.

Morphological Analysis. For the Gen_1 population of 138, there were 97 fertile and 41 sterile offspring (Table 11, Appendix). Eighty-six of the fertile plants produced height-segregating Gen_2 lines. Eleven of the fertile plants produced height-uniform Gen_2 offspring. For example, off-type 118 in Table 11 (Appendix) produced 9 offspring (B, D, F, G, I, J, M, N, and O) that produced height-segregating Gen_2 offspring, 3 offspring (C, E and K) that produced height-uniform Gen_2 offspring and 5 offspring (A, H, L, P and Q) that were sterile. Offspring analysis off 118 suggests the tall off-type selection was monosomic 4B and it produced 9 height-segregating monosomic 4B, 3 height-uniform disomic 4B and 5 Sterile nullisomic 4B offspring.

Table 1. Segregation of tall off-type offspring from Hi-Line semidwarf spring wheat. Generation 1 (Gen₁) was grown under greenhouse conditions in 1991, and Generation 2 (Gen₂) was grown under field conditions in 1992.

Offspring generation†	Offspring n	-----%-----		
		Monosomic 4B	Disomic 4B	Nullisomic 4B
Gen ₁	138	62	8	30
Gen ₂	1215	72	8	20
Gen ₁ + Gen ₂	1353	71	8	21

†Gen₁ plants were classified based on segregation of Gen₂ families. The Gen₂ classifications were based on phenotypes as described in Materials and Methods.

From the 1215 plants in Gen₂ segregating field-rows which were derived from tall off-type selections, 879 were consistent with the monosomic 4B phenotype, 95 were consistent with the disomic phenotype and 241 were sterile and consistent with the nullisomic 4B phenotype (Table 11, Appendix). The frequency of 0.21 nullisomic 4B offspring from a monosomic 4B Hi-Line parent (Table 1) was higher ($P < 0.05$) than the frequency estimate of 6.40% for Chinese Spring wheat by Sears (1954). The analysis of Gen₁ and Gen₂ offspring of off-type selections revealed cytogenetic conditions of the selections and segregation frequencies of offspring.

Our data suggest the frequency of 0.20% tall off-types in Hi-Line spring wheat is due to the loss of one chromosome

4B. Because the height-reducing gene, *Rht1*, is located on chromosome 4B, the loss of a chromosome 4B results in tall plants due to the *Rht1* locus being hemizygous. The off-type selections produced offspring that segregated as 0.71 monosomic 4B, 0.08 disomic 4B and 0.21 nullisomic 4B (Table 1). Tall off-types persist in each generation despite efforts to cull since the aneuploid condition occurs randomly. If tall off-types are retained in a population, the monosomic 4B predominantly produces monosomic 4B offspring (Table 1). After several succeeding generations, the population of tall off-types would peak at 0.69% by generation 20, according to calculations based on frequencies of monosomics derived from disomics and monosomics. These calculations are confirmed using a "sum to infinity" equation described by Riley and Kimber (1961).

The frequency of monosomy in wheat may vary for each chromosome and for different varieties (Morrison, 1953; Riley and Kimber, 1961; Sears, 1953 and 1954; Worland and Law, 1985). In most cases, aneuploidy is unlikely to result in an obviously altered phenotype. However, our results in combination with those of Worland and Law (1985) suggest that the monosomic condition of chromosomes 4B and 4D, containing the *Rht1* and *Rht2* genes, respectively, may lead to tall plants. Tall off-types may adversely affect grower acceptance and seed certification of varieties that produce tall off-types. Wheat breeders may consider using

alternative height-reducing genes or educating growers and certification agencies that tall off-types may be an inherent and unavoidable characteristic of certain semidwarf wheat varieties.

THREE

OCCURRENCE AND EFFECT OF
TALL OFF-TYPES IN WHEATLiterature Review

Wheat breeders work to develop genetically uniform cultivars. Despite the development of uniform cultivars, there has historically been an occurrence of phenotypic off-types due to outcrossing, mechanical mixtures and aneuploid conditions (Hollingshead, 1932; Love, 1943; Myers, 1938; Powers, 1932; Riley and Kimber, 1961; Thompson and Robertson, 1930). A concern has been the prospect of aneuploid conditions causing off-types. Whereas mechanical mixtures and outcrosses are permanently culled if phenotypically distinguishable, aneuploids may regularly occur in each generation, and some may occur as phenotypic off-types.

A wheat breeding program that seeks to minimize a particular off-type caused by an aneuploid condition requires genetic variation for occurrence and effect of a particular aneuploid condition. A few investigations have approached the question of genetic influence for aneuploid conditions between wheat genotypes. These studies concurred that different wheat varieties produced a frequency of pollen cells with abnormal chromosome numbers (Hollingshead,

1932; Myers, 1938; Powers, 1932; Riley and Kimber, 1961).

However, there are different results on the question of genotypic variance for the occurrence of aneuploidy.

Hollingshead (1932) analyzed the chromosome constitutions of pollen cells from 5 varieties and indicated the proportion of cells with univalents varied from 2.9 to 9.7%. Riley and Kimber (1961) analyzed 5 different varieties and indicated they had the same proportion of 6% cells with univalents.

Using the same varieties, Riley and Kimber (1961) tested for the transmission of aneuploid conditions to sporophytes and estimated an average frequency of 0.0108 aneuploidy; the highest proportion, 0.007, were monosomics, and no genotypic variance was detected. There is no conclusive evidence for or against genotypic variation for aneuploid conditions.

Studies that compare genotypes estimate average frequencies of aneuploid conditions for all chromosomes. Worland and Law (1985) suggest there is significant variation between chromosomes for frequencies of monosomic conditions, and Sears (1954) suggests there is variation between monosomic plants for the production of nullisomic offspring. These studies suggest some chromosomes have more propensity for loss, and particular aneuploid conditions may be more prevalent.

The question of genotypic variance for phenotypic effects has been superficially explored. Riley and Kimber (1961) discuss the different effects of monosomic conditions

between two varieties of wheat - 'Chinese Spring' and 'Holdfast'. These differences were considered examples of genotypic variance for phenotypic effects of monosomic conditions. Worland and Law (1985) cytologically analyzed phenotypic off-types in a population of 'Brigand' and showed that 77% of the off-types were aneuploids. About 60% of the aneuploids were monosomic 4D, which grew an average of 21% taller than the population mean. The monosomic 4D condition of Brigand was compared with the monosomic 4D condition of another variety, 'Hobbit', which grew an average of 11% taller than the population mean. The height differences caused by the same monosomic conditions were speculated to be a result of different genetic interactions.

Storlie and Talbert (1993) approached the question of tall off-types in a population of 'Hi-Line' spring wheat and showed that a frequency of 0.20% tall off-types were due to the loss of one chromosome 4B. A monosomic 4B condition resulted in the height-reducing *Rht1* locus being hemizygous which caused the wheat to grow between 17 and 34% taller than the population mean. Chromosome 4D contains a gene, *Rht2*, that confers gibberellin insensitivity and is involved with height reduction in some semidwarf and dwarf wheats (Gale et al., 1975). Another gene, *Rht1*, on chromosome 4B, serves a similar function (Gale and Marshall, 1976). The frequency and phenotype of the monosomic 4B condition in Hi-Line was a concern because growers and seed certification

agencies may consider the off-types a result of heterozygosity.

The objectives of this study were to determine if variation for occurrence and effect of tall off-types exists within and between *Rht1* and *Rht2* genotypes. Variation within *Rht1* or *Rht2* genotypes would suggest an effect of background genes; variation between *Rht1* and *Rht2* genotypes would suggest an effect of the gene and propensity for chromosome loss.

Materials and Methods

Varieties and *Rht* Genotypes

Semidwarf varieties selected for tall off-type analysis were based on availability and information on genotypes. Two height-reducing genes were selected for analysis, *Rht1* and *Rht2*. "*Rht1*" and "*Rht2*" are used to represent the genotypes, *Rht1Rht1rht2rht2* and *rht1rht1Rht2Rht2*, respectively. The *Rht* genotypes of 'Newana' and 'Pondera' were determined by reciprocal crosses with varieties containing known height-reducing genes. Hi-Line was previously documented as homozygous for *Rht1* (Storlie and Talbert, 1993). Kihara (1983) documented 'Siete Cerros', 'Inia' and 'Nacozari' as homozygous for *Rht1* and 'Ciano' and 'Era' as homozygous for *Rht2*. 'Len', 'Grandin' and 'Gus' were cited as homozygous for *Rht1* and 'McNeal', 'Marshall' and 'Wheatan' as homozygous for *Rht2* (L.E. Talbert, personal

communication).

Varietal Populations

Each of the 14 varieties noted above were planted with a 30.48 cm-spaced, 6-row planter at the Post Research farm (45° N, 110° W) on May 20, 1993. The field conditions included 624 mm of precipitation, a mean temperature of 5.04 °C and 113 frost free days in 1993. The field was fertilized with 190 Kg ha⁻¹ nitrogen. Variable quantities of seed for some varieties caused a range of population sizes. In most cases, varieties were planted in twelve 3.04 m ranges. About twenty seeds per 30.4 cm was estimated. In cases of small seed quantities, there were fewer ranges.

Tall Off-type Selections. On September 8, 1993, at maturity the population sizes and average heights were estimated. In each of 5 ranges, whole plants were removed from four 30.4 cm sections, counted and measured from the base, above the crown. Population densities of 30.4 cm sections were multiplied by row length to estimate population sizes. All tall individuals were selected in each variety based on heights that were above the tallest height group of a euploid population height distribution (Figure 6). The spike from the tallest tiller was harvested.

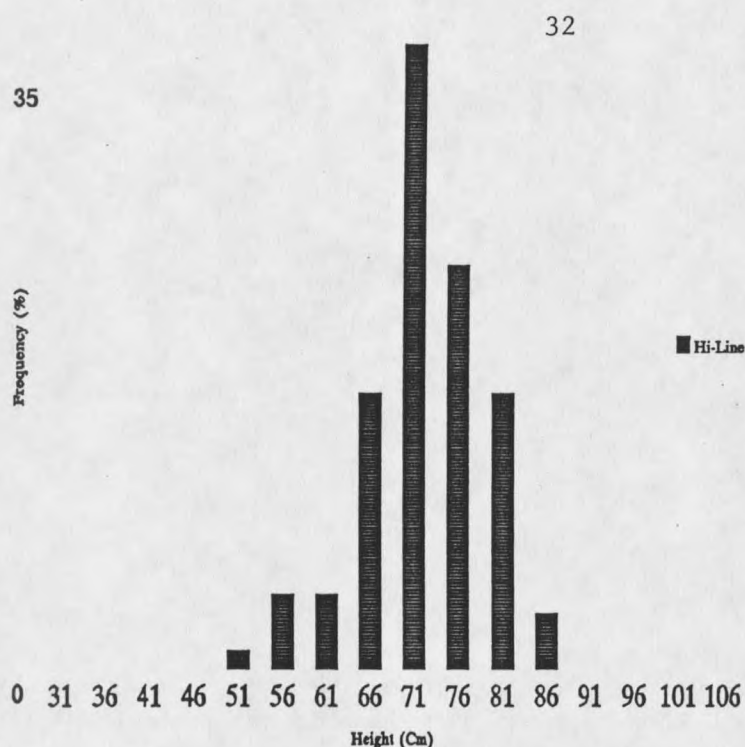


Figure 6. Height-distribution of a euploid semidwarf wheat, in 1991. Tall selections were plants that had heights above respective euploid distributions.

Tall Off-type Analysis. A seed from each of the tall selections was evaluated for its glutenin banding pattern using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by the method of Laemmli (1970) to determine genotypic purity.

Gen₁ offspring were grown in the greenhouse for an evaluation of height segregation, occurrence of nullisomic offspring and for production of Gen₂ offspring for a field evaluation, in 1993. Limited greenhouse space did not permit planting of all offspring from tall selections. Seven seed (Gen₁ offspring) of about 20 tall selections from each variety were planted in the greenhouse in 1.5" diameter

conetainers, December 1993. The tallest selections were prioritized for a greenhouse planting and the remaining selections were stored for a field planting. The greenhouse environment consisted of 21.1 °C day and night temperatures and 15 h days. Conetainer soil was saturated with water each day and on alternate days with 26 gL⁻¹ Peter's nutrient solution (Milpitas, CA).

After 1 month of growth, leaf tissue samples were removed from 46 Gen₁ plants for DNA extraction and PCR analysis. Samples were taken from offspring of several varieties of tall off-type selections. Sample selection was based on the plant phenotype, which may indicate a nullisomic 4B or 4D condition if the plant is dwarfed and relatively less vigorous (Storlie and Talbert, 1993).

PCR analysis using G10 primers was used to screen the offspring of tall off-type selections for nullisomic 4B or 4D DNA banding patterns. Using primer G10 on euploid wheat DNA produces 3 bands of amplified DNA when reactions are digested with *HinfI*. One of the bands is absent when nullisomic 4A, 4B or 4D DNA is amplified (Figure 7). These polymorphisms allow the identification of nullisomic group 4 plants. A frequency of nullisomic offspring produced from a tall off-type indicates a monosomic 4B or 4D condition for the off-type (Storlie and Talbert, 1993). The Gen₁ offspring were harvested in April, 1994; a spike was removed and retained individually for a Gen₂ analysis in the field.

Gen₁ and Gen₂ seed was planted at the Post Farm on May 10, 1994. The mean temperature for the growing season was 14 °C and total precipitation, from January to August, was 24 cm. Gen₁ and Gen₂ offspring of each tall selection were sown in a single row for evaluation of height-segregation and identification of plant morphologies that indicate nullisomic 4B or 4D conditions. Gen₂ field-rows represent offspring from Gen₁ plants grown in the greenhouse. The chromosomal constitution of each Gen₁ plant should be

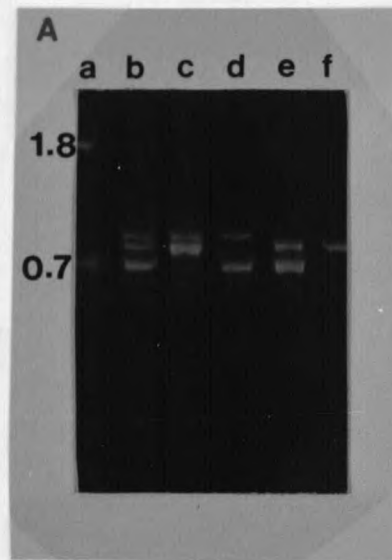


Figure 7. PCR reaction products of primer G10 used to amplify sequences on euploid and aneuploid wheat. Lanes a, pUC19 digested with *Rsa*I (1.8 and 0.7 Kb fragments); b, euploid Hi-Line; c, nullisomic 4D CS (Chinese Spring); d, nullisomic 4B CS; e, nullisomic 4A CS; f, euploid *Triticum speltoides*.

revealed by the Gen₂ offspring grown in the field (Storlie and Talbert, 1993). Height-segregation and an occurrence of nullisomic offspring in the two generations indicated a

monosomic condition for the tall selection. A height-uniform row indicated a euploid condition. Each row of offspring was evaluated beginning with the seedling stage of growth. Phenotypes that indicated a lack of vigor and a possible nullisomic condition were selected for morphological observations through maturity and DNA extraction for PCR analysis. After plants had grown and bolted, each row of offspring was measured for comparison with the height distribution of a euploid population.

Cytological Analysis

A sample of tall selections was examined for chromosome numbers to assess the reliability of height-segregations and PCR analyses for inference of monosomic conditions. Root tip squashes followed the smear technique of Tsuchiya (1971).

Statistical Analysis

Tall monosomic height comparisons were analyzed using One-way Analysis of Variance for contrasts (ψ_1); a standard error was estimated from a pooled variance of the cultivars. Contrasts compared height means of *Rht1* and *Rht2* genotypes and means within each genotype. Height variation of monosomic offspring was compared with respective variation of euploids using the *F* Test. Tall monosomic frequencies and offspring distributions were compared using the *chi-square statistic* (χ^2).

Results and Discussion

Genotype Determination

The F_2 populations of Newana/*rht1rht1Rht2Rht2* genotypes and Pondera/*rht1rht1Rht2Rht2* genotypes did not segregate for plant height. The height distributions of Newana/Era and Pondera/Pavon show normal, nonsegregating populations (Figures 8 and 9). The F_2 populations of Newana/*Rht1Rht1rht2rht2* genotypes and Pondera/*Rht1Rht1rht2rht2* segregated for height. The height distributions of Newana/Siete Cerros and Pondera/Siete Cerros show segregating populations (Figures 8 and 9). These results indicate Newana and Pondera are homozygous for *Rht2* which is on chromosome 4D.

Tall Selections

Populations of the 14 varieties ranged in size from 879 plants of Inia to 8016 plants of Grandin (Table 2). The number of talls selected from each variety ranged from 5 in Nacozari to 78 in Grandin (Table 3). A wide range of talls were investigated to estimate minimal heights caused by monosomic 4B and 4D conditions.

Exclusion of Three Varieties. Three varieties were excluded from analysis because of problems that may confound the results. Gus was removed due to mixing of seed during the harvest of tall selections. Marshall was removed due to

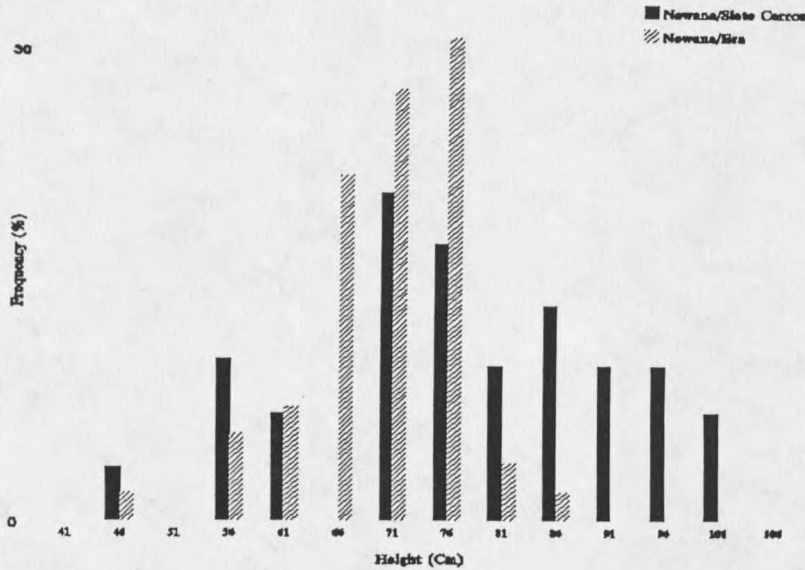


Figure 8. Height distributions of F2 Newana/Siete Cerros (genotype - *Rht1Rht1rht2rht2*) and Newana/Era (genotype - *rht1rht1Rht2Rht2*).

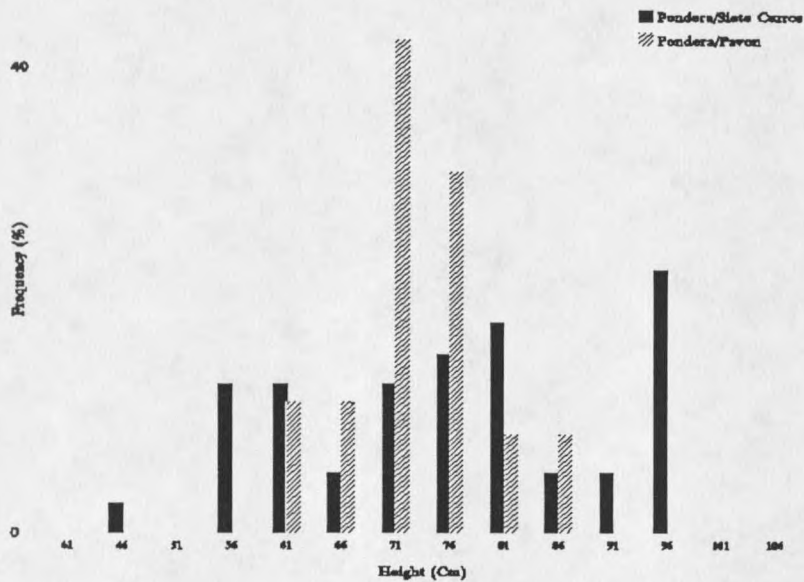


Figure 9. Height distributions of F2 Pondera/Siete Cerros (genotype - *Rht1Rht1rht2rht2*) and Pondera/Pavon (genotype - *rht1rht1Rht2Rht2*).

Table 2. Tall selections from 13 varieties with *Rht1* or *Rht2* genotypes harvested in 1993 from respective populations with size n and height $\bar{x}$.

Variety	<i>Rht</i> Genotype	Population n	Euploid height $\bar{x}$ cm ($n = 200$)
Grandin	<i>Rht1</i>	8016	93
Hi-Line	<i>Rht1</i>	4305	83
Inia	<i>Rht1</i>	879	84
Len	<i>Rht1</i>	7312	86
Nacozari	<i>Rht1</i>	1931	81
Siete Cerros	<i>Rht1</i>	2229	81
Ciano	<i>Rht2</i>	1208	79
Era	<i>Rht2</i>	5769	90
Marshall	<i>Rht2</i>	5245	84
McNeal	<i>Rht2</i>	7820	92
Newana	<i>Rht2</i>	7529	85
Pondera	<i>Rht2</i>	7449	89
Wheatan	<i>Rht2</i>	4814	90

Table 3. Tall selections from 13 varieties in 1993 with *Rht1* or *Rht2* genotypes measured for heights and genotypic purity:

Variety	<i>Rht</i> genotype	Tall selections <i>n</i>	Height range (%) above population $\bar{x}$	Percent pure genotypes
Grandin	<i>Rht1</i>	78	8-28	67
Hi-Line	<i>Rht1</i>	55	7-27	70
Inia	<i>Rht1</i>	12	7-20	92
Len	<i>Rht1</i>	60	5-22	81
Nacozari	<i>Rht1</i>	5	10-19	100
Siete Cerros	<i>Rht1</i>	32	10-23	100
Ciano	<i>Rht2</i>	15	3-34	60
Era	<i>Rht2</i>	36	10-21	83
Marshall	<i>Rht2</i>	33	7-30	55
McNeal	<i>Rht2</i>	47	5-17	100
Newana	<i>Rht2</i>	62	6-26	75
Pondera	<i>Rht2</i>	51	7-19	79
Wheatan	<i>Rht2</i>	15	15-25	67

an unusual occurrence of tall and nonsegregating offspring and the production of one sterile offspring that produced an unexpected nullisomic 4B banding pattern, rather than an expected 4D banding pattern. Marshall was thought to have the *Rht2* gene and is expected to produce a frequency of tall monosomic 4D wheat and a frequency of nullisomic 4D offspring. The nonsegregating tall offspring may be a result of outcrossing that was not detected by SDS-PAGE. Wheatan was removed due to the production of one sterile offspring that produced an unexpected nullisomic banding pattern. Wheatan, like Marshall, was thought to have the *Rht2* gene and is expected to produce a frequency of nullisomic 4D offspring. The unexpected nullisomic banding patterns in Marshall and Wheatan may be a result of an incorrect *Rht* classification, a sampling mistake or a process of monosomic shift (Person, 1956). The inclusion of an ambiguous genotype in this analysis may cause inaccurate comparisons of monosomic 4B and 4D frequencies for *Rht1* and *Rht2* genotypes.

Detection of Impure Genotypes. The tall selections were analyzed for glutenin banding patterns and compared with the variety banding pattern. Ten varieties had high levels of genotypic contamination, as indicated by percent purity in Table 3. For example, Figure 13 (Appendix) shows a gel consisting of the variety banding pattern for Hi-Line in lane b and for tall selection banding patterns in lanes

c-q. Samples that indicate contamination (similar to lanes h,i,j,k,l,m and o) were discarded. The percent genotypic purity of tall selections ranged from 100% in Nacozari, Siete Cerros and McNeal to 55% in Marshall (Table 3). Genotypic contamination was mostly due to outcrossing and mechanical mixing. Also, some polymorphism may be due to glutenin variation within the varieties. Mclendon et.al. (1993) evaluated some spring wheat varieties and indicated that Pondera was polymorphic and Era, Hi-Line and Newana were not polymorphic for glutenins.

Tall Monosomic Estimates

Comparisons between *Rht1* and *Rht2*. The results of the offspring segregation analysis infer that five varieties homozygous for *Rht1* and three homozygous for *Rht2* had tall off-types caused by monosomic 4B and 4D conditions, respectively (Table 12 a. and b., Appendix). One variety with the *Rht1* genotype, Inia, and 2 with the *Rht2* genotype, Ciano and Pondera, produced no detectable tall monosomics. The average frequencies of tall off-types in *Rht1* and *Rht2* populations were 0.0016 and 0.0005, respectively (Table 4). A χ^2 test indicates that more tall monosomic 4B wheat occurs from *Rht1* genotypes than tall monosomic 4D wheat occurs from *Rht2* genotypes ($P \leq 0.0001$).

Previous studies of tall off-types included analysis of varieties that are homozygous *Rht1* and *Rht2*, Hi-Line and

Table 4. Frequencies of tall monosomic 4B and 4D wheat compared between *Rht1* and *Rht2* genotypes of 11 varieties. This comparison includes all tall selections from Table 12.

Variety	<i>Rht</i> genotype	Population <i>n</i>	Monosomic 4B or 4D <i>n</i>	Monosomic 4B or 4D frequency
Grandin	<i>Rht1</i>	8017	9	0.0011
Hi-Line	<i>Rht1</i>	4305	10	0.0023
Inia	<i>Rht1</i>	879	0	0.0000
Len	<i>Rht1</i>	7312	9	0.0012
Nacozari	<i>Rht1</i>	1931	1	0.0005
Siete Cerros	<i>Rht1</i>	2229	3	0.0013
Ciano	<i>Rht2</i>	1208	0	0.0000
Era	<i>Rht2</i>	5769	3	0.0005
McNeal	<i>Rht2</i>	7820	6	0.0008
Newana	<i>Rht2</i>	7529	6	0.0008
Pondera	<i>Rht2</i>	7449	0	0.0000
Total	<i>Rht1</i>	20368	32	0.0016
Total	<i>Rht2</i>	29775	15	0.0005***

****Rht2* genotypes are significantly different from *Rht1* genotypes at 0.001 probability level ($\chi^2 = 14.70$, $P = 0.0001$).

Brigand, respectively (Worland and Law, 1985; Storlie and Talbert, 1993). This study includes seven more varieties (excluding Hi-Line and varieties that did not produce tall monosomic wheat) and implies that an occurrence of tall wheat caused by monosomic conditions is a common event. The results also indicate variability for the occurrence of monosomic 4B and 4D chromosomes, which concur with the results of Worland and Law (1985). The varieties Inia and Ciano did not produce detectable tall monosomics in relatively small population sizes - the size (n) of Inia was 879 and Ciano was 1208. Larger populations are needed for an accurate estimate of tall monosomic frequencies, because the occurrence of monosomic conditions is a random event.

Comparisons within *Rht1* and *Rht2*. The frequencies of tall monosomic wheat between *Rht1* genotypes were the same as the average of 0.0016 in Table 4 ($P > 0.05$). The frequencies between *Rht2* genotypes were the same as the average of 0.0005 in Table 4 ($P > 0.05$). These results provide evidence against genetic variation between varieties with the same *Rht* genotypes for the occurrence of tall monosomic wheat. Previous studies indicate an occurrence of aneuploid conditions may be, in part, heritable (Powers, 1932; Myers, 1938; Riley and Kimber, 1961). A problem with detecting variation in this study may be the differential population sizes of some varieties. An experiment designed to detect genotypic variation may provide more conclusive

information on heritable influences.

Frequencies of tall monosomics are based on offspring height-segregation and production of nullisomic plants, as described in materials and methods. A segregation ratio of 73 monosomics: 24 disomics: 3 nullisomics was theoretically calculated for offspring of a monosomic wheat (Sears, 1953, 1954). Storlie and Talbert (1993) suggest monosomic 4B Hi-Line produced 8 disomic 4B: 71 monosomic 4B: 21 nullisomic 4B. A frequency of each condition was expected to appear in the samplings of offspring rows for monosomic selections. For example, Hi-Line selection H (Table 12 a.) produced 3 Gen_1 plants which produced height-segregating Gen_2 offspring in field rows and 1 Gen_1 plant which produced a height-uniform field row. A total of 27 selection H offspring segregated in Gen_1 and Gen_2 , and 1 nullisomic 4B plant was detected among segregating offspring for a frequency of 0.037 nullisomic offspring (Table 12 b.).

Table 12 (a. and b.) list each tall off-type selection that segregated for height and produced nullisomic 4B or 4D plants. These selections were considered either monosomic 4B or 4D. The height-segregating offspring of these selections were grouped according to variety to compare height distributions with respective euploid populations. Height-segregating offspring produced skewed distributions (Figure 10), which exemplifies the high proportion of monosomics and height variation.

For each variety, height-segregation of monosomic offspring may be expressed as more height variance than respective euploid populations (Table 5).

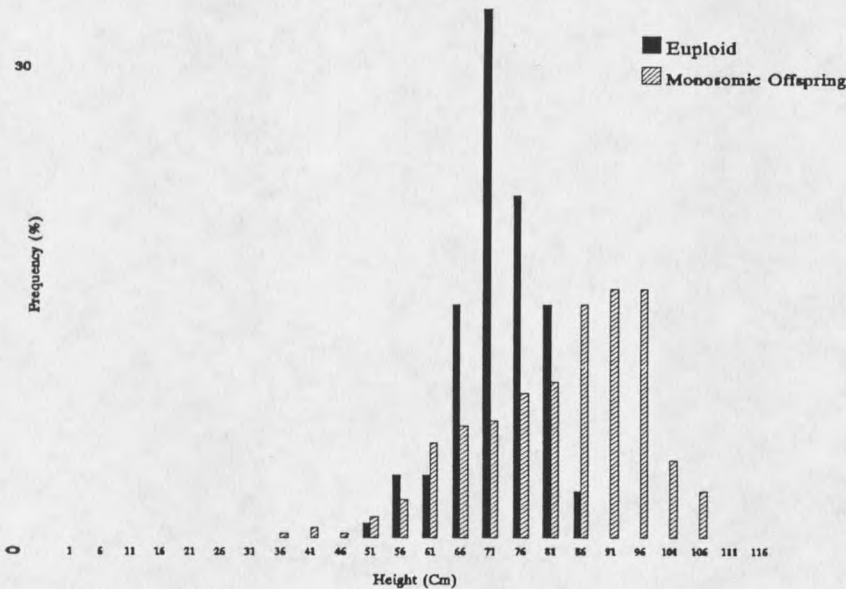


Figure 10. Height distributions of uniform euploid and segregating monosomic offspring of Hi-Line wheat, in 1994.

The occurrence of nullisomic offspring was determined by PCR, as described in materials and methods. Reactions of 16 Gen_1 offspring are shown in Figure 11. Four samples show nullisomic 4D or 4B banding patterns of offspring produced from tall selections - Era N, Grandin A and J and Siete Cerros E in Lanes e, f, l and o, respectively. Offspring of tall selections with nullisomic 4B and 4D morphologies were analyzed using PCR. An association of a nullisomic banding pattern with at least one offspring from Gen_1 or Gen_2 of each

Table 5. Height-segregating monosomic offspring and a height-uniform euploid population compared with respective height SD.

Variety	<i>Rht</i> type	Monosomic and euploid offspring	<i>n</i>	Height $\bar{x}$	Height SD
Grandin	<i>Rht1</i>	Monosomic 4B	241	89	7.9
		Euploid	50	82	5.5**
Hi-Line	<i>Rht1</i>	Monosomic 4B	110	82	9.3
		Euploid	40	70	6.4**
Len	<i>Rht1</i>	Monosomic 4B	109	84	11.1
		Euploid	52	75	6.5**
Nacozari	<i>Rht1</i>	Monosomic 4B	33	70	12.0
		Euploid	54	68	3.8**
Siete Cerros	<i>Rht1</i>	Monosomic 4B	100	71	10.5
		Euploid	49	68	4.4**
Era	<i>Rht2</i>	Monosomic 4D	100	80	7.7
		Euploid	56	76	4.7**
McNeal	<i>Rht2</i>	Monosomic 4D	226	84	7.1
		Euploid	43	78	4.4**
Newana	<i>Rht2</i>	Monosomic 4D	140	73	11.50
		Euploid	60	72	4.2**

**Euploid population has significantly less variation than monosomic offspring at 0.01 probability level using *F* Test.

selection was considered important evidence for an inference of monosomy and identification of the missing chromosome. Nineteen tall selections in Table 12 (a) were also confirmed cytologically to produce aneuploid Gen_2 offspring (Table 13, Appendix), which indicates that the height-segregation and PCR analyses are reliable for inferences of monosomic 4B and 4D wheat. Seven tall selections did not provide molecular or cytological evidence in conjunction with evidence of height-segregating offspring to infer a monosomic condition - 3 selections of McNeal, 2 of Len and 2 of Newana. These selections were included as monosomics because offspring of these selections segregated for height and they showed genotypically pure banding patterns when analyzed by SDS-PAGE. Statistical comparisons did not change without these selections.

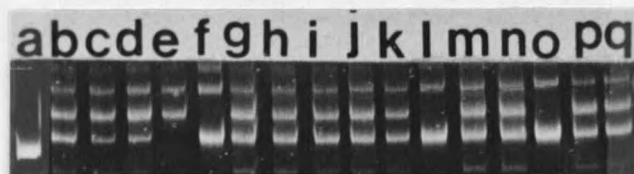


Figure 11. PCR used with primer G10 to screen the offspring of tall selections for nullisomic 4B and 4D banding patterns. Lanes a, pUC19 digested with *Rsa*I; b-q, tall off-type offspring. Lane e represents a nullisomic 4D offspring and lanes f, l and o represent nullisomic 4B offspring.

Phenotypic Effects of Monosomics

The heights of monosomic wheat were considered for an estimation of phenotypic effect between and within *Rht1* and *Rht2* genotypes. Monosomic 4B plants produced from varieties with *Rht1* produced taller heights than monosomic 4D plants produced from varieties with *Rht2* ($P = 0.012$). Tall monosomic 4B plants averaged 22% and ranged between 10 and 37% above respective population means (Table 6); tall monosomic 4D plants averaged 18% and ranged between 12% and 24% above respective population means (Table 6). Within *Rht1* genotypes, Hi-Line and Siete Cerros grew taller monosomic 4B plants than Len and Grandin ($P < 0.010$). Within *Rht2* genotypes, heights of monosomic 4D cultivars were the same ($P = 0.22$). These results suggest a monosomic 4B condition for *Rht1* genotypes has an increased height effect compared to a monosomic 4D condition for *Rht2* genotypes. The height differences within the *Rht1* genotypes suggest some genotypic influence on effect of tall monosomic 4B wheat.

Wheatan Albino Offspring. Two offspring of a tall Wheatan selection were white and completely lacking of green coloration. At the two leaf stage, each seedling was harvested and its DNA extracted for PCR with primer G10. Reactions representing each offspring are in Figure 12. Lanes h and i represent amplifications of albino wheatan and show nullisomic 4D banding patterns.

Table 6. Heights of tall monosomic 4B and 4D wheat compared for 8 varieties within and between *Rht1* and *Rht2* genotypes. Percentage values for height means above respective euploid populations for each cultivar are compared.

Variety	<i>Rht</i> genotype	<i>n</i>	Percent height $\bar{x}$ above population $\bar{x}$	Percent height range above population $\bar{x}$
Grandin	<i>Rht1</i>	10	18	11-25
Hi-Line	<i>Rht1</i>	10	28**	18-37
Len	<i>Rht1</i>	7	16	10-20
Nacozari†	<i>Rht1</i>	1	20	
Siete Cerros	<i>Rht1</i>	3	27**	25-30
Era	<i>Rht2</i>	3	17	12-21
McNeal	<i>Rht2</i>	7	17	15-20
Newana	<i>Rht2</i>	4	20	18-24
Average	<i>Rht1</i>	30	22	10-37
Average	<i>Rht2</i>	15	18*	12-24

**Rht1* genotypes had a significantly taller height effect than *Rht2* genotypes when monosomic ($P = 0.012$).

***Hi-Line and Siete Cerros were significantly different from other *Rht1* genotypes at the 0.01 probability level.

†Nacozari was excluded from statistical comparisons between *Rht1* and *Rht2* genotypes because of an occurrence of only 1 monosomic wheat.

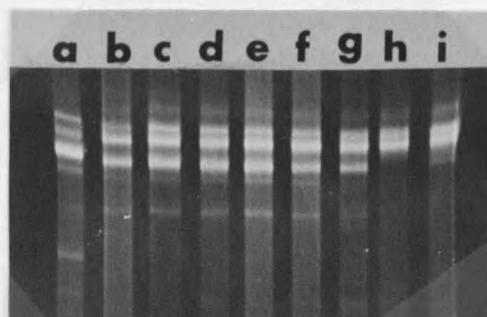


Figure 12. PCR analysis with primer G10 to screen the offspring of tall selections from Wheatan. Lanes a, euploid Chinese Spring; b-g, euploid Wheatan; h and i, albino Wheatan produce nullisomic 4D banding patterns.

The albino DNA was tested with primers specific for other chromosomes for an indication of other nullisomic conditions, and the results hint that other chromosomes may be absent, but these reactions were ambiguous, unlike the results provided by primer G10. The unusual phenotype is not explained by a nullisomic 4D condition, considering the usual green leaves of other nullisomic 4D wheat. A process of monosomic-shift, as explained by Person (1956), may produce a multiple monosomic plant which may produce offspring with an array of chromosomal constitutions and morphologies.

Monosomic Offspring

The offspring of tall monosomic wheat segregate as disomic, monosomic and nullisomic plants. The segregations of each tall selection are shown as proportions of monosomic

and disomic offspring (Table 12 a) and frequencies of nullisomic offspring (Table 12 b).

Comparisons between *Rht1* and *Rht2*. In Table 7, the proportion of 103 monosomic 4B to 29 disomic 4B offspring for *Rht1* genotypes was different than 69 monosomic 4D to 46 disomic 4D offspring for *Rht2* genotypes ($\chi^2 = 9.1$, $P = 0.003$). In Table 8, the frequency of 0.100 nullisomic 4B offspring for *Rht1* genotypes was different from 0.028 nullisomic 4D offspring for *Rht2* genotypes ($\chi^2 = 30$, $P = 0.0000$).

The analyses in Tables 7 and 8 indicate that monosomic 4B wheat produced a proportion of 70 monosomic 4B, 20 disomic 4B and 10 nullisomic 4B offspring, and monosomic 4D wheat produced a proportion of 58 monosomic 4D, 39 disomic 4D and 3 nullisomic 4D offspring. The results from Tables 7 and 8 indicate that monosomic 4B and 4D wheat produced different proportions of monosomic, disomic and nullisomic offspring. Compared with monosomic 4B wheat, varieties with monosomic 4D constitutions produced fewer monosomic and nullisomic offspring and more disomic offspring. A different trend occurred for comparisons of data from the cytological analysis of Gen_2 offspring (Table 13, Appendix). The proportions of offspring from monosomic 4B and 4D wheat did not differ ($\chi^2 = 1.2$, $P = 0.54$). Monosomic 4B and 4D wheat produced a proportion of 79 monosomic, 17 disomic and

Table 7. The total number of monosomic and disomic 4B or 4D offspring from tall monosomic selections. All selections from Table 12 are included for respective varieties.

Variety	<i>Rht</i> genotype	Monosomic 4B or 4D offspring†	Disomic 4B or 4D offspring‡
Grandin	<i>Rht1</i>	41	7
Hi-Line	<i>Rht1</i>	31	4
Len	<i>Rht1</i>	17	8
Nacozari	<i>Rht1</i>	5	2
Siete Cerros	<i>Rht1</i>	9	8*
Era	<i>Rht2</i>	13	7
McNeal	<i>Rht2</i>	33	23
Newana	<i>Rht2</i>	23	16
Total	<i>Rht1</i>	103	29
Total	<i>Rht2</i>	69	46**

*Segregation of Siete Cerros was significantly different from other *Rht1* genotypes ($\chi^2 = 5.0$, $P = 0.02$).

**Total segregation of *Rht1* genotypes was significantly different from *Rht2* genotypes ($\chi^2 = 9.1$, $P = 0.003$).

†Proportion of monosomics derived from monosomic wheat is totaled from all selections within each variety of Table 12.

‡Proportion of disomics derived from monosomic wheat is totaled from all selections within each variety of Table 12.

Table 8. Nullisomic 4B or 4D wheat that occurred per total offspring for all tall monosomic selections in Table 12.

Variety	<i>Rht</i> genotype	Proportion of nullisomic 4B and 4D per offspring	Nullisomic 4B and 4D frequency
Grandin	<i>Rht1</i>	19/358	0.053*
Hi-Line	<i>Rht1</i>	56/312	0.180*
Len	<i>Rht1</i>	7/223	0.031*
Nacozari	<i>Rht1</i>	4/38	0.105
Siete Cerros	<i>Rht1</i>	20/123	0.162
Era	<i>Rht2</i>	3/110	0.027
McNeal	<i>Rht2</i>	3/343	0.009
Newana	<i>Rht2</i>	15/282	0.053
Total	<i>Rht1</i>	106/1054	0.100
Total	<i>Rht2</i>	21/735	0.028***

*Grandin, Hi-Line and Len were significantly different from other *Rht1* genotypes ($\chi^2 = 4.9, 5.4$ and 4.7 , respectively, $P = 0.02, 0.02$ and 0.03 , respectively).

***Total of *Rht1* genotypes was significantly different from *Rht2* genotypes at the 0.001 probability level ($\chi^2 = 30$, $P = 0.0000$).

4 nullisomic offspring. The different proportions estimated for the cytological and height-segregation analyses may be due to the relatively small sample sizes used in the cytological analysis.

Monosomic 4B wheat produced the theoretical distribution of monosomic and disomic offspring estimated by Sears (1953) as, 75 monosomic: 25 disomic ($\chi^2 = 0.29$, $P = 0.59$). Monosomic 4D wheat produced the distribution of monosomic and disomic offspring estimated by Morrison (1953) as, 101 monosomic: 68 disomic ($\chi^2 = 0.001$, $P = 0.97$). These results indicate some correspondence with previous estimates of monosomic offspring. Segregation differences between *Rht1* and *Rht2* genotypes implicate chromosomes 4B and 4D as sources of variability, which may relate to differences in monosomic 4B and 4D frequencies in euploid populations.

Comparisons within *Rht1* and *Rht2* genotypes. In Table 7, monosomic Siete Cerros produced a proportion of 9 monosomic 4B to 8 disomic 4B offspring, which differs from the total for *Rht1* genotypes that produced a proportion of 103 monosomic 4B to 29 disomic 4B offspring ($\chi^2 = 5.0$, $P = 0.02$). In Table 8, monosomic Grandin, Hi-Line and Len produced nullisomic 4B offspring with frequencies of 0.031-0.180, which differs from the total for *Rht1* genotypes that produced nullisomic 4B offspring with a frequency of 0.10 ($\chi^2 = 4.9-5.4$, $P = 0.02-0.03$). These results indicate some

genotypic influence on the production of monosomic 4B offspring. A genotypic influence on frequencies of chromosome loss may be expected to correspond between aneuploid conditions. For instance, the same genotypic influence on the occurrence of monosomic 4B plants may be expected to apply to the production of nullisomic 4B offspring from monosomic 4B plants. However there was no detection of a genotypic influence in the analysis of monosomic 4B and 4D occurrences (Table 4). As discussed before, these results may require further investigation to determine heritable influences.

Conclusion

Monosomic conditions are caused by bivalent pairing failure at meiosis, which occurs with probability for all chromosomes (Riley and Kimber, 1961). A difference in monosomic frequencies for the 21 chromosomes indicates some chromosomes may be more dispensible in the occurrence of aneuploidy. The only evidence for differences in chromosome loss is provided by Worland and Law (1985) for variance of monosomic occurrences, and Sears (1954) for frequencies of nullisomic offspring from monosomic plants; Sears (1954) suggests that monosomic 4B plants produce a higher frequency of nullisomic 4B offspring than monosomic 4D plants produce nullisomic 4D offspring. One objective of this investigation was to determine if *Rht1* and *Rht2* genotypes

produce different tall monosomic frequencies, a question based on a presumption of differences in chromosome loss.

This study analyzed 11 varieties which were selected on a basis of *Rht* genotype identification and availability. Six varieties were homozygous *Rht1* and 5 were *Rht2*. A comparison of tall monosomic frequencies indicate that varieties with *Rht1* and *Rht2* have frequencies of 0.0016 and 0.0005, respectively. Three varieties produced no tall monosomic wheat. In the instances of Inia and Ciano the lack of monosomic detection may be due to the relatively small population sizes (n). These data may be used to influence a breeding program with an inclination to reduce the occurrence of tall monosomic plants by incorporation of *Rht2* into semidwarf wheat cultivars.

Results of an analysis for variation within *Rht1* or *Rht2* genotypes indicate no genotypic influence. However, some relatively small population sizes (n) of varieties may have reduced the sensitivity of comparative tests. A conclusive study may indicate there are genotypic influences on the occurrence of monosomic 4B and 4D wheat.

The phenotypic effect of height for *Rht1* genotypes was greater than the effect for *Rht2* genotypes in this study. Heights were 22% and 18% above population means for cultivars with *Rht1* and *Rht2* genotypes, respectively. Some genetic background variation within *Rht1* genotypes was detected; monosomic 4B Hi-Line and Siete Cerros were

significantly taller than the total for *Rht1* genotypes. Genetic background influences on the effect of tall monosomics may be difficult to manipulate in a breeding program unless a method is developed to predict these effects in small early-generation populations.

Results of the segregation analysis of monosomic 4B and 4D offspring indicate that two proportions of monosomic, disomic and nullisomic wheat are produced: In Tables 7 and 8, monosomic 4B wheat produced a proportion of 70 monosomic 4B, 20 disomic 4B and 10 nullisomic 4B offspring; monosomic 4D wheat produced a proportion of 58 monosomic 4D, 39 disomic 4D and 3 nullisomic 4D offspring. The differences in segregation between monosomic 4B and 4D wheat may relate to the same causes of variability for frequencies of monosomic 4B and 4D wheat in Table 4.

Results of this study corroborate previous studies that suggest a frequency of tall off-types in wheat is caused by monosomic 4B or 4D conditions and hemizyosity for *Rht1* or *Rht2* (Worland and Law, 1985; Storlie and Talbert, 1993). The effect is a wheat plant that is 10-37% taller than a euploid semidwarf population mean. The problem of tall monosomics may be unavoidable, though results of this study indicate that variation for monosomic 4B and 4D conditions between *Rht1* and *Rht2* genotypes and for phenotypic effects between and within *Rht1* and *Rht2* genotypes may be utilized to minimize the occurrence and effect.

FOUR

DNA SEQUENCE ANALYSIS FOR ESTIMATION OF GENOME
RELATIONSHIPS IN TRITICUM AESTIVUMLiterature Review

The origin of *Triticum aestivum* ($2n=6x=42$) involved interspecific crosses of diploid species with A, B and D genomes (Kihara et al., 1959). Each genome has a diploid ancestor (Sears, 1966), which may be indicated by phylogenetic analysis. The A genome was derived from *T. monococcum* ($2n=2x=14$) (Fernandez de Caleyá et al., 1976; Gill and Kimber, 1974; Jones et al., 1982; Kerby, 1986; Kihara, 1919). The D genome was derived from *T. tauschii* ($2n=2x=14$) (Fernandez de Caleyá et al., 1976; Gill and Kimber, 1974; Jones et al., 1982; Kihara, 1944; McFadden and Sears, 1944, 1946; Pathak, 1940; Riley and Chapman, 1960). The B genome derivation is unclear though, in general, it was derived from a S-genome species within section *Sitopsis* (Kerby and Kuspira, 1987; Kimber and Sears, 1987). Initially chromosome pairing studies of crosses between *T. monococcum*, *T. turgidum* and *T. aestivum* revealed the three common wheat genomes (Kihara, 1919, 1924). Chromosome pairing studies have predominantly followed Kihara's "analyser-method" to determine genomic relationships in *Triticeae* (Kihara and Nishiyama, 1930). Kihara's method

relies on pairing relationships between diploids and polyploids to identify the polyploid genomes. The premise of chromosome pairing is that genomes with more DNA homology will preferentially pair (Alonso and Kimber, 1981), and homology as indicated by pairing relationships may provide insight into evolutionary relationships (Kimber and Feldman, 1987).

Pairing relationships of the three genomes in wheat have been studied for insight into closeness of relationships (Alonso and Kimber, 1982, 1983; Belfield and Riley, 1969; Dhaliwal et al., 1977; Hutchinson et al., 1983; Jauhar et al., 1991; Miller, 1981; Naranjo et al., 1987, 1988; Okamoto and Sears, 1962; Riley and Kempana, 1963). Assessing closeness of relationship between A, B and D is difficult because the B-genome donor is not known; relationship studies are bound to rely directly on the wheat genomes.

Early results conflicted between studies. Okamoto and Sears (1962) analyzed translocations in haploids and indicated most translocations were between homoeologous chromosomes of the A and D genomes. Belfield and Riley (1969) used telocentric stocks of Chinese Spring to cross with *Triticum speltoides* tausch. ($2n=14$) to observe meiosis for chromosome pairing and concluded there is no preferential pairing between genomes. These studies may have been problematic because chromosomes were not

identified with certainty (Hutchinson et al., 1983).

Recent studies have more accurately identified chromosomes, which has produced more congruent results. Alonso and Kimber (1983) used double-telocentric hybrids and mathematical models simulating chromosome pairing for results that indicate A and D genomes have more affinity than either A or D has with B. Hutchinson et al. (1983) used C-banding on wheat aneuhaploids and wheat-rye hybrids to identify chromosomes that paired at meiosis, and the results indicated that A and D chromosomes preferentially paired. Jauhar et al. (1991) used *ph1ph1* euploids and N-banded genomes in a study that indicated 80% of metaphase I pairing occurred between A and D genomes. Studies using chromosome pairing have been useful in establishing genomic classifications within *Triticeae*. A problem with classification using pairing analysis are conditions of partial pairing. Continuous variation caused by partial pairing may be difficult to compare (Kellogg, 1989).

Electrophoretic technologies have created possibilities of comparing genomes at the molecular level for more definitive classification and evolutionary distances (Crawford, 1990). Plant molecules used in systematic studies include seed storage proteins, Rubisco and isozymes, amino acid sequences, antigens, repetitive and single copy DNA, ribosomal DNA and chloroplast DNA (Crawford, 1990). Several molecular approaches have been used in studies of

the A, B, and D genome donors and wheat evolution (Anderson and Greene, 1989; Appels et al., 1980; Asins and Carbonell, 1986; Bahrman et al., 1988; Bowman et al., 1983; Caldwell and Kasarda, 1978; Chen et al., 1975; Dvořák et al., 1988; Forde et al., 1985; Halford et al., 1987; Harberd et al., 1987; Hsaiao et al., 1994; Hirai and Tsunewaki, 1981; Johnson, 1972, 1975; McIntyre, 1988; McNeil et al., 1994; Monte et al., 1993; Nath et al., 1983, 1984; Ogihara and Tsunewaki, 1982, 1988; Peacock et al., 1981; Sugiyama et al., 1985; Talbert et al., 1991; Thompson and Nath, 1986; Thompson et al., 1985; Tsunewaki and Ogihara, 1983).

Success using wheat proteins for phylogenetic analysis has varied. Studies involving seed storage proteins or Rubisco separation on polyacrylamide gels have provided inconsistent data on the origin of polyploid wheats (Crawford, 1990). Studies involving isozyme analysis (Asins and Carbonell, 1986; McIntyre, 1988) are considered to have drawbacks because the small number of loci generates too little data to properly reconstruct a phylogeny (Doebley and Wendell, 1989; Dvořák and Zhang, 1992). A study involving two-dimensional gel electrophoresis of section *Sitopsis* and Chinese Spring proteins implicated *T. speltoides* var. *aucheri* as a B-genome donor (Bahrman et al., 1988). This approach is considered to have advantages because of the large number of characters for phylogenetic analysis (Dvořák and Zhang, 1992).

The use of DNA for phylogenetic analysis is in its infancy as methods advance from less to more direct DNA manipulations. Studies involving DNA-DNA hybridizations indicate homology between *T. urartu* (currently classified as *T. monococcum* var. *urartu*) and the A genome (Nath et al., 1983, 1984 and Thompson and Nath, 1986). These results provoked a reclassification of *T. urartu* to an A-genome group. A study involving cpDNA restriction fragment analysis of 35 *Triticum* and *Aegilops* species was used to conclude *T. speltoides* was the donor of *T. turgidum* cytoplasm (Ogihara and Tsunewaki, 1988). Dvořák and Zhang (1992) stated the dendrogram generated by cpDNA data was in disagreement with dendrograms generated by chromosome pairing studies.

Studies involving the use of repetitive and low copy DNA clones for Southern blot analysis have the advantage of generating large numbers of characters for phylogenetic analysis (Dvořák and Zhang, 1992; Monte et al., 1993). Talbert et al. (1991) used repetitive DNA to analyze relations within *Triticum* section *Sitopsis* and indicated *T. speltoides* is distinct from other *Sitopsis* species, which is in agreement with several previous studies (Bahrman et al., 1988; Jaaska, 1978; Ogihara and Tsunewaki, 1988). Dvořák and Zhang (1992) used repetitive DNA to estimate relationships of 13 diploid *Triticum* species which had consistent data with many pairing relationship studies. Monte et al. (1993) used low copy cDNA clones for

restriction-fragment-length-polymorphism analysis to estimate evolutionary relationships among and within 16 species of *Triticeae* and concluded that data generated from this analysis were generally consistent with other morphological studies, chromosome pairing studies, isozyme tests and sequence alignments (Monte et al., 1993).

DNA sequence alignments may have the most potential of all methods of DNA analysis for estimating accurate phylogenies (Monte et al., 1993). Sequence comparisons in *Triticeae* include 5s DNA, Ter and Nor loci (for review, see Appels et al., 1989), ITS sequences of rDNA (Hsaiao et al., 1994), Dgas44 repetitive sequences (McNeil et al., 1994), high-molecular-weight glutenin genes (Anderson and Greene, 1989; Forde et al., 1985; Halford et al., 1987; Harberd et al., 1987; Sugiyama et al., 1985; Thompson et al., 1985). Anderson and Greene (1989) compared sequences of glutenin alleles from each of the genomes and aligned sequences from A and D genomes but could not align B because of different nucleotide repeats. These results suggest a closer relationship between the A and D genomes.

The objectives of the following study were to determine the degree of relatedness of homoeologous DNA sequences which were amplified by PCR using primer set G10 (Storlie and Talbert, 1993). Sequence comparisons may infer relationships of the A, B and D genomes, which may contribute to data from previous relationship studies and

answer some questions about the evolution of hexaploid wheat.

Materials and Methods

Plant Material and DNA extraction

One g of Chinese Spring leaf tissue was used for DNA extraction, according to method of Dellaporta (1983). Leaf tissue was ground in 15 mL extraction buffer - 100 mM Trishydroxymethylaminomethane (Tris), pH 7.6, 50 mM EDTA (Ethylenediaminetetraacetic acid, disodium salt dihydrate), pH 8.0, 100 mM NaCl, 1% SDS, 10mM β -mercaptoethanol - with mortar and pestle. The homogenized tissue was incubated in a 65 °C waterbath for 10 min. After incubation 5 mL 5 M potassium acetate ($\text{CH}_3\text{CO}_2\text{K}$) was added to homogenate and incubated on ice for 20 min. The homogenate was centrifuged at 25,000 x g for 20 min. The supernatant was poured through a miracloth filter into 10 mL cold isopropanol, $(\text{CH}_3)_2\text{CHOH}$, and 1 mL 5 M ammonium acetate ($\text{CH}_3\text{CO}_2\text{NH}_4$). The supernatant was mixed and incubated at -20 °C for 20 min and then centrifuged at 20,000 x g for 15 min to pellet the DNA. The DNA was resuspended in 0.7 mL TE (10 mM Tris and 1 mM EDTA) and precipitated in 75 μL 3 M sodium acetate ($\text{CH}_3\text{CO}_2\text{Na}$), pH 7.0, and 500 μL cold isopropanol. The DNA solution was gently mixed and centrifuged at 10,000 x g for 1 min to pellet the DNA . Finally the DNA was resuspended in 100 μL TE.

Polymerase Chain Reaction

Primers of 20 bases in length were designed from RFLP clones G10, and evaluated on Chinese Spring nullisomic group 4 stocks; primer design, analysis and PCR conditions and product analysis are described by Storlie and Talbert (1993). Three amplified DNA fragments were associated with chromosomes 4A, 4B and 4D.

Purification of the Amplified Fragment

The amplified DNA was purified using Prep-A-Gene DNA Purification Kit (Bio-Rad, Richmond, California). One hundred μL of PCR reaction product (approximately 1 μg amplified DNA) was added to 10 μL purification matrix and 330 μL binding buffer (6 M sodium perchlorate (NaClO_4), 50 mM Tris, pH 8, and 10 mM EDTA). Matrix solutions were mixed briefly and incubated for 10 min at RT with constant agitation. The matrix with DNA was centrifuged for 30 sec. The supernatant was discarded and the matrix resuspended in 500 μL binding buffer; the rinse with binding buffer was repeated 3 times. Subsequently, the rinsing procedure was repeated 3 times with wash buffer - 20 mM Tris, pH 7.5, 2 mM EDTA and 50% ethanol ($\text{C}_2\text{H}_5\text{OH}$). After the final rinse, the matrix pellet was dried in a Speed Vac (Savant, Farmingdale, NY), resuspended in an equal volume of elution buffer (10mM Tris, pH 8 and 1 mM EDTA) and incubated in a 37 °C waterbath for 5 min. Finally the matrix solution was centrifuged for

30 sec to separate the matrix and DNA; the pure DNA was removed with the supernatant.

Plasmid Preparation

Approximately 1 μ g of pUC19 was digested with 1 unit of *Sma*I in a 50 μ L reaction vol. at 25 °C for 1 h. Plasmid DNA was precipitated with 100 μ L ETOH and .25 M sodium acetate (pH 5.2). The DNA solution was placed on ice for 5 min. before centrifuging at 16,000 x g for 5 min. to pellet the plasmid DNA. The plasmid was dephosphorylated to prevent recircularization, as follows: approximately 0.5 μ g of DNA was suspended in 10 μ L of phosphatase buffer - 50mM Tris, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, threo-1,4-dimercapto-2,3-butanediol, (pH 7.9 at 25 °C), 1 μ L alkaline phosphatase calf intestinal (New England BioLabs, Beverly, MA) and incubated at 50 °C for 60 min. To terminate the reaction, EDTA was added to 5 mM and heated to 65 °C for 1 h. The dephosphorylated plasmid was purified with Prep-A-Gene kit as described previously.

Ligation and Transformation

The amplified DNA fragments were ligated to the dephosphorylated pUC19, according to the method of King and Blakesley (1986). Ligation conditions included 50 mM Tris (pH 7.6), 10 mM $MgCl_2$, 1 mM ATP, 170 ng (60 fmol) dephosphorylated pUC19, 13 ng (20 fmol) of amplified DNA, 1 unit T_4 DNA ligase (Gibco BRL, Gaithersburg, MD). Reactions

were mixed briefly, centrifuged for 5 s and incubated at RT (23 - 26 °C) for 12 h.

The procedure for transforming *E. coli* with plasmid DNA is outlined by Maniatis et al. (1982). A single colony of *E. coli* (strain, NM522) was used to inoculate 10 mL of LB (Luria-Bertani) medium (1.0% bacto trypton, 0.5% bacto yeast extract and 171 mM NaCl). The culture was incubated at 37 °C overnight with constant shaking at 150-200 rpm. The following day, 500 µL of the culture was used to inoculate a fresh 50 mL of LB medium in a side-arm flask and grown as above until an OD₆₀₀ of 0.40 to 0.60 was read on a photoelectric colorimeter (Klett-Summerson, NY). At this range of OD, cells had reached a density of about 3.2×10^8 - 4.8×10^8 cells/mL. Twenty-five mL of cell culture was decanted into each of two 30 µL Oak Ridge centrifuge tubes (Nalgene, Rochester, New York) and cooled for 5 min on ice before being centrifuged at 6000 x g for 5 min. The supernatants were discarded and the pellets combined and resuspended in 25 mL of 50 mM CaCl₂. The CaCl₂ solutions were mixed until the cells dispersed and placed on ice for 15 to 60 min before being centrifuged at 6,000 x g for 5 min. The supernatants were discarded and resuspended in 4 mL 50 mM CaCl₂. Glycerol was added to a final concentration of 15% of total vol. Competent cells were stored at -80 °C in 200 µL aliquots, after a brief immersion in liquid nitrogen.

Transforming the competent cells involved thawing on ice a 200 μ L aliquot of cells and adding 20 ng of ligated plasmid DNA. The cells and plasmids were briefly mixed and incubated on ice for 30 min, transferred to a 42 °C waterbath for 45-60 s and transferred again to ice for 1 min. Next, 3.7 mL of LB medium was added to the cell and plasmid mix and shaken at 37 °C for 45 min before being centrifuged and resuspended in 200 μ L of LB medium. LB plates with 100 μ g/mL ampicillin, 40 μ g/mL x-gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) and 0.5 mM IPTG, isopropyl thio- β -D-galactoside (Fisher, Pittsburgh, PA), were used to screen bacterial colonies for transformants, as described by Maniatis et al. (1982). The 200 μ L of transformed cells were spread on an LB plate and incubated at 37 °C O.N. After the incubation period, white colonies were selected as potential transformants with the desired clones. These colonies were picked with a sterile applicator stick and grown in a tube containing 750 μ L 2YT medium (1.6% bacto tryptone, 1.0% bacto yeast extract and 85 mM NaCl) with 2x ampicillin on a 37 °C shaker set for 150 RPM O.N. After growing O.N., 400 μ L glycerol was added to the culture and mixed before storing in a vial at -70 °C.

The clones were tested for expected sizes of DNA sequences using PCR to amplify the cloned inserts followed by electrophoresis of PCR products on a polyacrylamide gel for size estimation. The PCR reaction conditions were

modified slightly from the previous description. 1 μ L of stored cell culture was added to 49 μ L reaction mix, which had primer sequences designed to anneal to polylinker DNA of pUC19. The primer sequences are - Reverse, TTCACACAGGAAACAG and Universal, GTAAAACGACGGCCAGT. The thermocycling program was the same except the annealing temperature was 55 °C. The reaction products were separated by electrophoresis as previously described. The amplified cloned fragments were compared with sizes of amplified genomic fragments on polyacrylamide gels. PCR products produced from clones that were approximately the same size as products produced from genomic DNA were considered potential homoeologous G10 fragments.

DNA Sequencing

Plasmids from bacterial colonies with potential G10 sequences were purified according to the method described by Prep-A-Gene (Bio-Rad, Richmond, California). Cells were grown O.N. in 3 mL of 2YT containing 2x ampicillin at 250 rpm and 37 °C. After incubation, the cell cultures were centrifuged at 13,000 x g for 30 sec. The supernatant was decanted and the pellet resuspended in 200 μ L GET buffer, lysozyme solution (50mM glucose, 25 mM Tris, pH 8, 10 mM EDTA) and 400 μ L of alkaline lysis solution (0.2 N NaOH, 1% SDS). The cell solution was inverted and placed on ice for 2 min before adding 300 μ L of cold (4 °C) 7.5 M ammonium

acetate, pH 7.5, and again the solution was inverted and placed on ice for 5 min. The lysed cells were centrifuged at 13,000 x g for 5 min at RT and the supernatant decanted into new microcentrifuge tubes with 0.6 Vol. of isopropanol. The tubes were inverted and placed at RT for 2 min to precipitate DNA. The precipitate was centrifuged at 16,000 x g for 5 min to pellet the DNA; the supernatant was discarded and the pellet was air dried for 1 min. The DNA pellet was resuspended in 100 μ L of 2 M ammonium acetate, pH 7.5, and placed on ice for 5 min before centrifuging at 16,000 x g for 2 min. The supernatant was decanted into new tubes and the plasmid DNA was purified using a Prep-A-Gene kit as described previously for DNA purification.

Sequencing Reaction. Sequencing reactions followed the instructions of Sequenase Version 2.0 DNA sequencing kit (USB, Cleveland, Ohio). Approximately 1.25 pmole (3 μ g) plasmid DNA was denatured in 0.2 M NaOH and 0.2 mM EDTA solution at 37 °C for 30 min. The DNA was precipitated with 3 M sodium acetate (pH 4.8) and 4x volume ETOH and a -70 °C incubation for 20 min. The precipitate was centrifuged for 30 min at 4 °C to pellet the DNA. The supernatant was discarded and the pellet dried in a vacuum centrifuge at 45 °C and 12,800 x g for 10 min. The DNA pellet was resuspended in 14 μ L H₂O and divided as 7 μ L into 2 microcentrifuge tubes; one tube was for the -40 primer and

the other for the Universal primer reactions. The -40 and universal primer sequences are GTTTTCCCAGTCACGAC and GTAAAACGACGGCCAGT, respectively.

The first reaction involved annealing of primers to template. Two μL reaction buffer (200 mM Tris-HCl, pH 7.5, 100 mM MgCl_2 , and 250 mM NaCl) and 1 μL (0.5 pmole) of either -40 or Universal primer were added to each of the tubes with plasmid suspended in 7 μL Vol. Annealing reactions were incubated at 37 °C for 30 min and subsequently stored on ice.

The labeling reaction included the annealed primer and template and 7 mM DTT, 0.2 μM each of dGTP, dTTP and dCTP, 5 μCi [α - ^{35}S] dATP and 3 units Sequenase enzyme in a total Vol. of 15 μL . The reaction mix was incubated at 20 °C for 5 min. The termination reactions involved the use of individual tubes for each dideoxy nucleotide and for each primer. Each termination reaction included 3.5 μL of labeling reaction mix and 2.5 μL of appropriate dideoxy termination mixture (80 μM dGTP, dATP, dTTP, dCTP and 8 μM of either ddGTP, ddATP, ddTTP or ddCTP. The termination reaction was incubated at 37 °C for 5 min before 4 μL stop solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF) was added to repress the sequenase.

Gel Electrophoresis. The sequencing gel contained 6% acrylamide, 7 M urea, 1x TBE buffer (0.09 M Tris, 0.09 M boric acid, 20 mM EDTA), 0.5 mM ammonium persulfate and 3.5 mM Temed (N,N,N',N'-tetramethylethylenediamine) and was formed in a Bio-Rad Sequencing Apparatus (Bio-Rad, Hercules, California). The gel was heated to 50 °C in 1x TBE buffer and the sequencing reactions were denatured at 90 °C before being loaded onto the gel. Each reaction was loaded with a micro-syringe in respective lanes. The sequences were separated on the gel at 50 °C with a current of about 30 mA. After about 2 h of electrophoresis, the gel was transferred onto 3 mm chromatography paper (Whatman, Maidstone, England) and dried on a gel dryer (Hoefer Scientific Instruments, San Francisco, CA). The dried gel was placed on diagnostic film in an X-ray Exposure Holder (Kodak, Rochester, NY) and the film was exposed O.N. The film was developed using procedures outlined by Maniatis et al. (1992). The exposed film was read for DNA sequence from the bottom, beginning with a known polylinker sequence.

Sequence Analysis

Sequences were aligned using the Genepro program (Riverside Scientific Enterprises, Seattle, WA), which utilizes an algorithm developed by Needleman and Wunsch (1970). Parsimony analysis was conducted by Matt Lavin (Montana State University) on the aligned sequences with a

barley sequence as an outgroup using Hennig 86 (Ferris, 1988), which includes a Bootstrap and Decay Index analysis. Distance matrix analysis was conducted by Matt Lavin on the aligned sequences with a barley sequence as an outgroup using Phylip (Felsenstein, 1993), which utilizes Fitch and Margoliash's (1967) method.

Results

The DNA sequences amplified with primer G10 of the A, B and D genomes of Chinese Spring wheat are aligned with the barley sequence in Figure 14 (Appendix). These data were used for a phylogenetic analysis to estimate genome relationships with barley as an ancestral outgroup.

Parsimony Analysis

Parsimony analysis of the 4 DNA sequences built phylogentic trees from 9 phylogenetically informative characters (Table 9). Six character sites required 1 nucleotide substitution and 3 sites required 2 substitutions to produce a most parsimonius tree. This tree grouped the 4A and 4D genomes on a separate clade from the B genome. Two clade stability tests were conducted for an indication of robustness. A bootstrap analysis indicated that 93% of random character site analysis produced a separate clade for the A and D genomes. A decay index indicated that the most parsimonious tree with a different topology, that did not group the A and D genomes separately, would require 8

Table 9. Phylogenetically informative characters of G10 amplified sequences from barley and wheat genomes.

Genome	Informative Sites†
4A	CCACGTCCC
4B	GAACCCTTT
4D	CCCAGTTCC
Barley	GACACCCTT

†Phylogenetically informative sites allow for two or more trees. In this analysis each site has two nucleotides and two taxa share one of the nucleotides, (Stewart, 1993).

additional nucleotide substitutions.

Distance Matrix Method

The Distance Matrix method estimated the number of nucleotide substitutions per site between each pairwise comparison of the genomes (Table 10). This analysis estimated the 4A and 4D genomes to have the fewest nucleotide substitutions and consequently a closer evolutionary distance. Two clade stability tests were conducted to determine the robustness. An average percent standard deviation of 4.65 was estimated for six alternative topologies to the one in Table 10. A bootstrap analysis indicated that 61% of a random sequence analysis produced a separate clade for the A and D genomes.

Table 10. Pairwise comparisons of the wheat - A, B and D - and barley genomes. Comparisons are estimates of nucleotide substitutions per site.

Genome	4B	4A	4D	Barley
4B	0.0000			
4A	0.0752	0.0000		
4D	0.0796	0.0691	0.0000	
Barley	0.1379	0.1331	0.1621	0.0000

Conclusion

These results corroborate several relationship studies that indicate the A and D genomes are more closely related than either are to the B genome (Alonso and Kimber, 1983; Anderson and Greene, 1989; Hutchinson et al., 1983; Jauhar et al., 1991; Okamoto and Sears, 1962). The current study builds on previous relationship studies by the use of homologous DNA sequences from each of the genomes of wheat and barley. In both Parsimony and Distance Matrix methods of analysis, the B genome was grouped separate from a clade that contained the A and D genomes. The evolutionary relationship of the B genome in relation to the A, D and barley genomes could not be determined from the data used in this analysis. The lack of statistical robustness in this study may be improved with more character sites - more sequence data - which may increase the confidence of the A and D genome clade and estimate a relationship for the B

genome. A clarification of relationships of the wheat genomes and diploid ancestors is important for understanding wheat evolution and may be important for wheat breeders to utilize ancestral germplasm for intraspecific crosses.

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APPENDIX
Research Data

Table 11. Offspring of tall selections evaluated for segregation as monosomic, disomic and nullisomic wheat for an indication of the chromosomal constitution of the selection.

Tall selection	Gen ₂ n	Monosomic 4B	Disomic 4B	Nullisomic 4B
118 B	22	17	3	2 †
118 D	5	4	0	1
118 F	18	14	1	3
118 G	20	16	1	3
118 I	21	17	2	2
118 J	22	16	3	3
118 M	26	22	1	3
118 N	22	17	1	4
118 O	24	15	3	6
118 C	15	0	15	0 ‡
118 E	16	0	16	0
118 K	19	0	19	0
118 A	0	st		§
118 H	0	st		
118 L	0	st		
118 P	0	st		
118 Q	0	st		
113 D	10	6	1	1
113 K	12	7	1	3
113 L	20	15	2	3
113 M	26	16	4	6
113 N	38	29	3	6
113 P	21	15	1	4

Table 11. Continued.

Tall selection		Gen ₂ n	Monosomic 4B	Disomic 4B	Nullisomic 4B
113	Q	15	11	1	3
113	R	15	10	2	3
113	F	42	se		9
113	A	0	st		
113	B	0	st		
113	C	0	st		
113	E	0	st		
113	G	0	st		
113	H	0	st		
113	I	0	st		
113	J	0	st		
113	O	0	st		
211	A	10	8	0	2
211	B	35	33	2	6
211	C	22	15	1	6
211	E	17	12	1	4
211	G	9	7	1	1
211	H	5	5	0	0
211	I	23	16	2	5
211	J	2	2	0	0
211	K	16	8	2	6
211	L	5	2	0	3

Table 11. Continued.

Tall selection	Gen ₂ n	Monosomic 4B	Disomic 4B	Nullisomic 4B
211 M	14	9	0	5
211 P	16	11	3	2
211 R	11	9	1	1
211 U	17	8	3	5
211 N	18	0	18	0
211 D	0	st		
211 F	0	st		
211 O	0	st		
211 Q	0	st		
211 S	0	st		
211 T	0	st		
213 H	14	10	1	2
213 O	33	24	3	6
213 P	16	11	2	3
213 R	13	7	2	4
213 S	22	16	0	5
213 A	18	se		
213 C	21	se		
213 F	23	se		
213 G	10	se		
213 I	15	se		
213 J	21	se		
213 K	18	se		

Table 11. Continued.

Tall selection		Gen ₂ n	Monosomic 4B	Disomic 4B	Nullisomic 4B
213	L	22	se		
213	M	14	se		
213	Q	12	se		
213	D	19	0	19	0
213	B	0	st		
213	E	0	st		
213	N	0	st		
213	T	0	st		
307	B	24	18	2	4
307	C	7	6	1	0
307	F	20	16	0	1
307	K	19	12	2	4
307	M	27	20	2	5
307	N	20	16	2	2
307	O	26	20	3	3
307	Q	22	21	0	1
307	E	16	se		
307	G	21	se		
307	J	20	se		
307	L	22	se		
307	P	24	0	24	0
307	A1	0	st		
307	A2	0	st		

Table 11. Continued.

Tall selection		Gen ₂ n	Monosomic 4B	Disomic 4B	Nullisomic 4B
307	D	0	st		
307	H	0	st		
307	I	0	st		
307	R	0	st		
314	A	15	7	3	5
314	C	2	2	0	0
314	D	21	16	2	3
314	E	18	11	2	5
314	F	8	4	0	4
314	H	9	6	0	3
314	I	12	4	2	6
314	J	11	7	0	4
314	K	7	5	0	2
314	L	24	18	1	5
314	M	20	14	2	4
314	B	25	0	25	0
314	G	0	st.		
317	A	20	16	0	2
317	D	23	15	2	4
317	F	8	7	1	0
317	H	29	19	1	9
317	I	17	15	1	1
317	J	31	16	3	12

Table 11. Continued.

Tall Selection	Gen ₂ n	Monosomic 4B	Disomic 4B	Nullisomic 4B
317 B	0	st		
317 C	0	st		
317 E	0	st		
317 G	0	st		
317 K	0	st		
319 A	4	2	0	2
319 B	20	15	0	5
319 D	15	10	2	3
319 G	23	16	1	6
319 I	20	14	1	4
319 M	17	15	0	2
319 O	12	6	2	4
319 Q	24	17	3	4
319 S	15	13	1	1
319 N	12	se		
319 F	14	0	14	0
319 L	25	0	25	0
319 P	19	0	19	0
319 R	22	0	22	0
319 C	0	st		
319 E	0	st		
319 H	0	st		
319 J	0	st		
319 K	0	st		

†Gen₂ offspring segregated as indicated under monosomic 4B, disomic 4B and nullisomic 4B columns. ‡Gen₂ offspring did not segregate and were disomic 4B. §Tall selection was a sterile nullisomic 4B. ¶Gen₂ offspring were only determined to segregate and were not quantified.

Table 12. a.) Tall monosomic selections produced a proportion of monosomic and disomic offspring which were determined by analyzing Gen₁ and Gen₂ offspring.

Variety	Tall monosomic selection	<i>Rht</i> genotype	Monosomic 4B or 4D†	Disomic 4B or 4D‡
Hi-Line	H	<i>Rht1</i>	3	1
	I		2	0
	N		4	1
	O		5	0
	Y		4	0
	B1		3	1
	C1		6	0
	D1		4	1
	K1		-	- §
	L1		-	-
Grandin	D	<i>Rht1</i>	5	1
	I		6	1
	J		6	0
	O		3	1

Table 12. a.) Continued.

Variety	Tall monosomic selection	<i>Rht</i> genotype	Monosomic 4B or 4D†	Disomic 4B or 4D‡
Grandin	T	<i>Rht1</i>	6	1
	E1		6	1
	G1		3	1
	E		-	-
	O1		-	-
Len	H	<i>Rht1</i>	3	3
	T		7	0
	R		4	3
	A1		3	2
	D		-	-
	N		-	-
	X		-	-
	O1		-	-

Table 12. a.) Continued.

Variety	Tall monosomic selection	<i>Rht</i> genotype	Monosomic 4B or 4D†	Disomic 4B or 4D‡
Len	Q1	<i>Rht1</i>	-	-
Nacozari	C	<i>Rht1</i>	5	2
Siete Cerros	B1	<i>Rht1</i>	4	1
	D1		3	4
	E1		2	3
Era	G	<i>Rht2</i>	6	1
	Q		4	3
	N1		3	3
McNeal	U	<i>Rht2</i>	3	3
	X		3	4
	B1		3	3
	C1		5	2

Table 12. a.) Continued

Variety	Tall monosomic selection	<i>Rht</i> genotype	Monosomic 4B or 4D†	Disomic 4B or 4D‡
McNeal	A	<i>Rht2</i>	4	2
	Z		3	3
Newana	Q	<i>Rht2</i>	3	3
	V		4	2
	X		5	2
	A1		4	3
	W		2	3
	S		-	-

Pondera ¶

†Monosomic proportions were estimated from Gen_1 offspring that produced height-segregating Gen_2 offspring.

‡Disomic proportions were estimated from Gen_1 offspring that produced height-uniform Gen_2 offspring.

§Selections do not have estimates for monosomic and disomic proportions.

¶Monosomic wheat was not detected.

Table 12. b.) Tall monosomic selections produced a proportion of nullisomic offspring which were determined by analyzing Gen₁ and Gen₂ offspring.

Variety	Tall monosomic selection	<i>Rht</i> genotype	Nullisomic 4B or 4D per segregating offspring†	Nullisomic 4B or 4D frequency
Hi-Line	H	<i>Rht1</i>	1/27	0.037
	I		1/18	0.055
	N		8/41	0.195
	O		12/53	0.226
	Y		10/36	0.277
	B1		5/28	0.178
	C1		10/50	0.200
	D1		5/26	0.192
	K1		3/13	0.230
	L1		1/20	0.050
Grandin	D	<i>Rht1</i>	1/40	0.025
	I		1/46	0.022
	J		2/48	0.042
	O		2/25	0.080

Table 12. b.) Continued.

Variety	Tall monosomic selection	<i>Rht</i> genotype	Nullisomic 4B or 4D per segregating offspring†	Nullisomic 4B or 4D frequency
Grandin	T	<i>Rht1</i>	2/51	0.040
	E1		5/42	0.119
	G1		4/13	0.307
	E		1/9	0.111
	O1		1/8	0.125
Len	H	<i>Rht1</i>	1/20	0.050
	T		1/62	0.016
	R		0/58	0.000
	A1		0/28	0.000
	D		1/15	0.066
	N		1/14	0.071
	X		1/7	0.143
	O1		1/5	0.200
	Q1		1/14	0.071

Table 12. b.) Continued.

Variety	Tall monosomic selection	<i>Rht</i> genotype	Nullisomic 4B or 4D per segregating offspring†	Nullisomic 4B or 4D frequency
Nacozari	C	<i>Rht1</i>	4/38	0.105
Siete Cerros	B1	<i>Rht1</i>	7/44	0.160
	D1		11/47	0.234
	E1		2/32	0.062
Era	G	<i>Rht2</i>	1/54	0.018
	Q		1/29	0.034
	N1		1/27	0.037
McNeal	U	<i>Rht2</i>	0/34	0.000
	X		0/34	0.000
	B1		1/35	0.028
	C1		2/43	0.046
	A		1/35	0.028

Table 12. b.) Continued.

Variety	Tall monosomic selection	<i>Rht</i> genotype	Nullisomic 4B or 4D per segregating offspring	Nullisomic 4B or 4D frequency
McNeal	Z	<i>Rht2</i>	0/28	0.000
Newana	Q	<i>Rht2</i>	3/21	0.143
	V		7/48	0.146
	X		4/46	0.062
	A1		1/46	0.022
	W		0/34	0.000
	S		0/12	0.000

Pondera‡

†Nullisomic wheat that occurred in the total number of segregating Gen_1 and Gen_2 offspring.

‡Monosomic wheat was not detected.

Table 13. Tall selections confirmed cytologically produced Gen_2 offspring that were monosomic, disomic and nullisomic.

-----n-----					
Variety	Tall monosomic selection	<i>Rht</i> genotype	Disomic†	Mono- somic‡	Nulli- somic§
Grandin	T	<i>Rht1</i>	0	5	0
	D		2	1	0
	E1		0	6	1
	J		0	5	0
	O		2	1	0
Hi-Line	O1	<i>Rht1</i>	0	2	0
	Y		1	3	0
	K1		0	2	0
	L1		0	1	1

Table 13. Continued.

-----n-----

Variety	Tall monosomic selection	<i>Rht</i> genotype	Disomic†	Mono- somic‡	Nulli- somic§
Len	N	<i>Rht1</i>	4	4	1
	T		0	7	0
	X		0	8	0
Siete Cerros	B1	<i>Rht1</i>	0	3	0
Era	Q	<i>Rht2</i>	0	4	0
McNeal	B1	<i>Rht2</i>	1	3	0
Newana	V	<i>Rht2</i>	3	10	1
	X		2	3	0
	A1		1	3	0
Total		<i>Rht1</i>	9	48	3
Total		<i>Rht2</i>	7	23	1 NS

†Number of Gen₂ offspring with 42 chromosomes.‡Number of Gen₂ offspring with 41 chromosomes.§Number of Gen₂ offspring with 40 chromosomes.

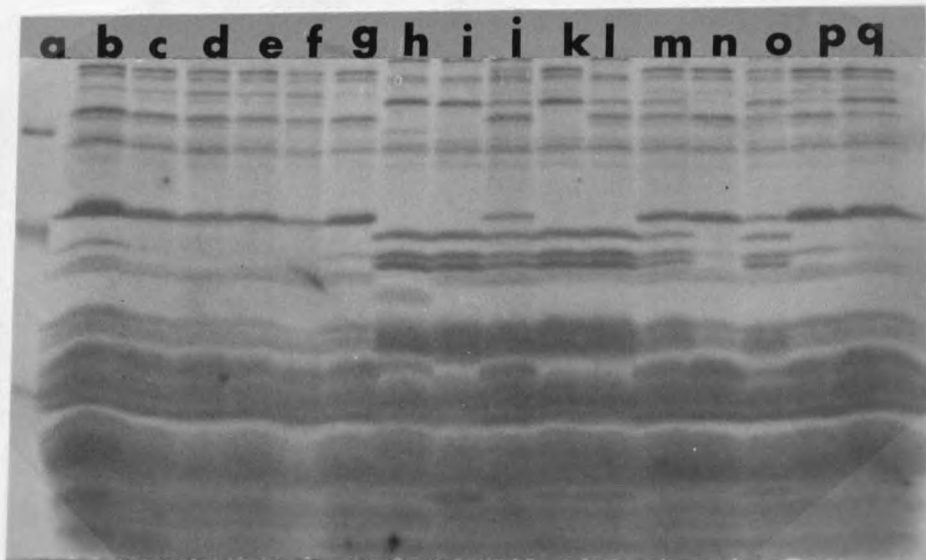


Figure 13. SDS-PAGE of glutenins for analysis of genotypic purity of tall selections in a population of Hi-Line. Lanes a, molecular weight standard - 45, 66 and 97.4 kD; b, euploid Hi-Line; c-q, tall selections. Lanes c-g show uniform banding patterns and lanes h-q show some genotypic contamination.

Figure 14. Aligned DNA sequences of PCR products from primer G10. Products were isolated from barley and chromosomes 4A, 4B and 4D of wheat.

<u>Chromosome</u>	<u>1</u>	<u>Nucleotides</u>	<u>56</u>
#4B	GGCCAACCTCGTGGGTCATGAACTTGTCCAGCTCCAGCTCCTGTGT		
#4A	GGCCGACCTCGTGGGTTATGAACTTGTCCAGCTCCAGCTCCTGTCA		
#4D	GGCCAACCTCGTGGGTTATGAACTTATCCAGCTCCACCTCCT-ACA		
#Barley	GGCCAACCTCGTGAGTTATGAACTTGTCCAGCTCCAGCTCCTGTGG		

<u>Chromosome</u>	<u>57</u>	<u>Nucleotides</u>	<u>103</u>
#4B	TGTCC-----AACAAAACAAAAACACATGGGAAAATTCTCTGAATC		
#4A	TGTCCAAATAGACCACAACAC-----ATGTAAAAAT-CTCTGAATC		
#4D	-----A-----AACAAAACAC-----ATGGAAAATTCTCTGAATC		
#Barley	CGTCC-----AACAAAACAAAAACACATAGAAAATTCTCTGAATC		

<u>Chromosome</u>	<u>104</u>	<u>Nucleotides</u>	<u>150</u>
#4B	AGAAGCTCTCAAGAACATCG-CAGTGCTACGCATGGTCAGTCTGATG		
#4A	AAAACCTCTC--GAACATCGA-----CAGTCTGATG		
#4D	AGAAGCTCTC--GAACATCGACAGTGCCACGCATGGTCAGTCTGATG		
#Barley	AGAAGCTCTCTCGAACATCG-GCAATACACACGAT--CGGTCTGACG		

<u>Chromosome</u>	<u>151</u>	<u>Nucleotides</u>	<u>195</u>
#4B	AGTGTACACCTTGTTTCAGGTACTTGTCCGGCAGGATTGGAATGTC-		
#4A	AGTGTACACCTTGTTTCAGGTACTTGTCCGGCAGGA-CGGGATGTC-		
#4D	AGTGTACACCTTGTTTCAGGTACTTGTCCGGCAAGGATCGGAATGTC-		
#Barley	GGTGTACACCTTGTTTCAGGTACTCGTCCGGCGCTCGTCCGGCGTGTCC		

Figure 14. Continued.

<u>Chromosome</u>	<u>196</u>	<u>Nucleotides</u>	<u>239</u>
#4B	TGCTTGGGCTTCACCCCCTCGAAGAGCGACCCCATGACGCATTT		
#4A	TGCTTGGGCTTCACGCCTCCGAAGAGCGACCCCATGACGCACTT		
#4D	TGCTTGGGCTTCCAGCCTCCGAATTGCGATCCCATTACGCATTT		
Barley	TGCTTGGGCTT-CACCCCGCGAAGAGA--CCCCATAACGCACTT		

<u>Chromosome</u>	<u>240</u>	<u>Nucleotides</u>	<u>279</u>
#4B	ACCGTGGTGGACGTGACCCAGGTCGTCAAGGTCGACCCCG		
#4A	ACCGTGGTGGACGTGACCCAGGTCGTCAAGGTCGCCCCCG		
#4D	ACCGTGGTGGACGTGACCCAGGTCGTCAAGGTCGACCCCG		
#Barley	ACCGTGGTGGACGTGACCCAGGTCGTCAAGGTCGACCCCG		

<u>Chromosome</u>	<u>280</u>	<u>Nucleotides</u>	<u>319</u>
#4B	CCGTGCCGCCGCGCCACCGCCTGCCTCCTCAGCTGCGGCGC		
#4A	CCGTGCCGCCGCGCCACCGCCTGCCTCCTCAGCTGCGGCGC		
#4D	CCGTGCCGCCGCGCCACCGCCTGCCTCCTCAGCTGCGGCGC		
#Barley	CCGTGCCGCCGTCCAC-GCCTGCCTCCTCAGCTGCGGCGC		

<u>Chromosome</u>	<u>320</u>	<u>Nucleotides</u>	<u>342</u>
#4B	CACCACCGGTCAGTCCACTACAC		
#4A	CACCACCGGTCAGCACACC-CAC		
#4D	CACCACCGGTCAGCACACC-CAC		
#Barley	CACCACCGGTCAGTGCACT-CGC		

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