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Mapping of beta-tubulin genomic sequences in hexaploid oat (*Avena sativa* L.)

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Summary. The allohexaploid nature of *Avena sativa* L. (2n = 6x = 42) and the availability of aneuploid lines was exploited in designing a strategy for mapping beta-tubulin sequences in the oat genome. Evidence for a minimum of eight beta-tubulin genes was obtained by Southern-blot analysis. Three beta-tubulin sequences were localized to chromosomes using DNA from monosomic and nullisomic lines in the variety Sun II. One sequence was localized to the chromosome missing in nullisome I. Two other sequences were mapped to satellite chromosome 2, the chromosome that is missing in nullisome VIII and to which one ribosomal RNA gene cluster had previously been mapped. Restriction fragments carrying these two beta-tubulin genomic sequences and the cluster of ribosomal RNA sequences were missing in DNA from nullisomics VIII, IX and X, suggesting that all three nullisome classes are deficient for an identical chromosomal segment that includes these three loci. This study demonstrates how molecular analyses can be used to characterize aneuploid stocks and to better define their genetic constitution.

Key words: Mapping – Beta-tubulin – Monosomic – Nullisomic – *Avena sativa* L.

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Introduction

One strategy for mapping DNA sequences to the chromosomes of polyploid species relies on the possibility of isolating viable monosomic and nullisomic lines. DNA from an inbred disomic (2n) plant would contain two copies of a sequence that is localized on a particular restriction enzyme fragment. DNA from a specific monosomic (2n – 1) plant would contain only one copy of the sequence whereas DNA from the corresponding nullisomic (2n – 2) plant would be deficient for both copies of the sequence and would lack the restriction fragment. We employed this strategy to map molecular markers to chromosomes of the allopolyploid species *Avena sativa* L., using available viable monosomic and nullisomic lines.

Moderately repetitive sequences which are dispersed in the genome provide valuable hybridization probes for genomic mapping studes because multiple copies of the sequence can be mapped simultaneously on the same set of hybridization filters. One such sequence is beta-tubulin which is encoded by small multigene families ranging in size from two to 20 members in most higher eukaryotes examined to-date (reviewed by Cleveland and Sullivan 1985; Silflow et al. 1987). Tubulin genes encode proteins of approximately 50 kDa which form dimers with alpha-tubulin for assembly into microtubules, the filamentous polymers that constitute a major component of the cytoskeleton in eukaryotic cells. Multiple beta-tubulin genomic sequences have been observed in several plant species including *Brassica napus*, *B. oleracea*, *Nicotiana tabacum*, *Glycine max*, *Pisum sativum*, *Triticum aestivum*, and *Zea mays* (Guiltinan et al. 1987; Marks et al. 1987; Hussey et al. 1990). The most complete analysis has been carried out in *Arabidopsis thaliana* which has nine

beta-tubulin genes, all of which are expressed (Snustad et al. 1992). Tubulin genes are clustered in the genomes of some protozoan species (reviewed by Cleveland and Sullivan 1985) and tandem arrangements of pairs of duplicated tubulin genes have been found in *Z. mays* and *A. thaliana* (Oppenheimer et al. 1988; Montoliu et al. 1989). However, in most species examined, the majority of tubulin genes have been found dispersed throughout the genome.

In the present study the number of beta-tubulin genes in the hexaploid genome of oat has been determined. The conserved nature of the tubulin coding region permitted many members of this gene family to be analyzed on a single Southern blot hybridized with a coding region probe. The strong possibility that the tubulin genes would be dispersed in the oat genome suggested that individual beta-tubulin sequences could serve as molecular markers for different chromosomes in mapping studies.

Materials and methods

Oat plants

Seeds of the cultivar 'Sun II' and of the monosomic and nullisomic stocks in a 'Sun II' background were obtained from H. Thomas of the Welsh Plant Breeding Station, Aberystwyth, Wales. The monosomics and nullisomics had been recovered primarily as naturally occurring variants identified by cytological screening of root tips of seedlings in varietal populations. From studies of the phenotype and, in some cases, the karyotype of the monosomics and their derived nullisomics, the monosomics had been placed into 15 classes designated by Roman numerals based on the order of recovery (Hacker and Riley 1965; H. Thomas, personal communication). These classes were considered to be deficient for different chromosomes; however, because only three of the nullisomics were fertile (VII, XI and XIV), it was not possible to determine genetically whether the different classes were distinct.

Plants of 12 monosomic and three fertile nullisomic classes were grown in the greenhouse and allowed to self. The resulting seeds from the monosomic lines were used to screen for segregating disomics, monosomics and nullisomics, which occurred in varying frequencies depending on the monosomic class. Segregating monosomic plants, which are fairly normal in phenotype in most classes and are produced at frequencies of 29–93% in progeny from their respective monosomic parents (Hacker 1965), were presumptively identified by the characteristic occurrence of a high frequency of micronuclei in quartet microspores. Immature panicles were fixed and stored as described by Rines and Johnson (1988). Microsporocytes were stained with either acetocarmine or propionocarmine. Micronuclei were counted in at least 25 microspore quartets. Vigorous plants with few micronuclei-containing quartets (< 5%) were classified as disomics. Special emphasis was placed on collecting tissue from the weaker, smaller plants segregating usually at low frequencies. These were considered possible nullisomics but they often did not develop floral tissue adequate for meiotic chromosome analysis. Nullisomic material from 11 classes (I, II, IV, VI, VII, VIII, IX, X, XI, XII, and XIV) was recovered for further analysis. Leaf tissue was collected from tillers of 5–6-week old plants for DNA extraction.

Hybridization probes

Two oat beta-tubulin cDNA clones, designated β1 and β3, were used as hybridization probes (Mendu et al. 1985). Both clones were isolated from a cDNA library prepared from RNA isolated from the cultival 'Garry' (Hershey et al. 1984) and were shown to encode beta-tubulin by DNA sequence analysis (Mendu 1990). A 1250-bp *Pst*I fragment of the β1 cDNA (EMBL Accession number X 54852) and a 1300-bp *Pst*I fragment of the β3 cDNA were both cloned into M13mp19 for use as coding region probes. The 5′ (440 bp) and the 3′ (610 bp) non-overlapping probes were generated from the β1 coding region. Single-strand phage DNA was prepared and radio-labeled by primer extension (Hu and Messing 1982).

The *Z. mays* rDNA probe was obtained from S. Enomoto and I. Rubenstein (University of Minnesota); the plasmid pZmr1 contains a repeat of the maize 9-kb ribosomal RNA genes including the intergenic region. This plasmid was radiolabeled by nick translation.

Genomic DNA isolation

The isolation of DNA from leaf samples (0.5–2.0 g) was carried out by the method of Dellaporta et al. (1983). After precipitation with isopropanol, the nucleic acid pellet was resuspended in 100 µl of TE (50 mM Tris-HCl pH 8.0, 10 mM EDTA). Contaminating carbohydrates were removed by adsorption to cellulose CF-11 (Mozer 1980). Five hundred µl of cellulose slurry [Whatman CF-11, 10% (w/v) prepared as described by McClure and Guilfoyle 1987] and 25 µl of 3 M NaOAc was added to the DNA solution which then was mixed and centrifuged briefly to sediment the cellulose. Approximately 200 µg of DNA was recovered from the supernatant by precipitation with ethanol.

Southern blots and hybridization

Genomic DNA (20 µg) was digested with 100 units of restriction enzyme in the buffer specified by the manufacturer, 5 mM spermidine, and 1 µg RNase A. The DNA fragments were electrophoretically separated on 0.8% agarose gels (5–6 mm thick, 22 cm long) in Tris-acetate buffer (40 mM Tris, 2 mM EDTA, 5 mM NaOAc pH 8.0) at 30 V for 24 h. The buffer was recirculated during electrophoresis. The DNA was either depurinated by acid treatment (0.25 N HCl, 8 to 10 min) or nicked by UV irradiation for a total of 1.2 joules in the 'Stratalinker' (Stratagene, La Jolla, Calif.). After denaturation with alkali and neutralization as described by Southern (1975), the DNA was transferred to a nylon membrane (Genatran, Plasco Inc., Woburn, Mass.) by capillary action, using the transfer solution of Rigaud et al. (1987) with modifications (1 M NH_4OAc, 1 M NH_4OH, 0.2 M NaOH). The membrane was washed in 2 × SSC (0.3 M NaCl, 30 mM Na-Citrate) and baked at 80 °C for 2 h.

The filters were incubated for 12 to 24 h, at 42 °C, with prehybridization solution [5 × SSPe (0.9 M NaCl, 50 mM $NaPO_4$ pH 7.7, 5 mM EDTA), 50% formamide, 50 mM Tris-HCl pH 7.5, 1 x Denhardt solution (0.2% each of ficoll, polyvinylpyrrolidone, bovine serum albumin), 1% SDS (Sigma), 0.1 mg/ml sheared, denatured calf thymus DNA]. The solution was replaced with hybridization solution (the prehybridization solution plus 10% Dextran sulphate and 0.5–0.75 µg of radioactive DNA probe), using 0.5 ml of solution per cm^2 of membrane. Hybridization was carried out at 42 °C for 24 h. The filters were washed with 0.2 × SSC, 0.2% SDS at 62 °C for 2–3 h. Autoradiographs were obtained by exposure of the membranes to XAR-5 (Kodak) film, with or without intensifying screens, for 16–48 h.

Filters were stripped of the radioactive probe by first immersing the membrane in 0.4 N NaOH for 40 to 120 min at

42 °C, followed by neutralization in a solution of 1 M Tris-HCl pH 7.5, 0.2 × SSC, 0.2% SDS at room temperature.

Results

The oat genome contains multiple beta-tubulin sequences

Genomic DNA from *A. sativa* cv 'Garry' was digested with one of three different restriction enzymes, fractionated on agarose gels and then transferred to nylon membranes. Hybridization of the DNA with complete coding regions from two different oat beta-tubulin cDNA clones (probes designated a and d in Fig. 1) resolved 12 to 15 hybridizing fragments, depending on the enzyme used to digest the DNA (Fig. 2A). To determine which of these fragments contained a complete beta-tubulin coding sequence, the blots were stripped and rehybridized with non-overlapping probes from either the 5′ (designated b in Fig. 1A) or the 3′ (designated c in Fig. 1A) region of the tubulin coding sequence. The probes hybridized to subsets of the DNA fragments identified by the complete coding region probes (Figs. 2B and C). Depending on the enzyme used, 5–8 different fragments hybridized to both of these probes (asterisks in Fig. 2A), suggesting that the fragments contained a complete coding sequence. Based on these data, we estimate that the oat genome contains a minimum of eight complete beta-tubulin genes. Additional DNA fragments that hybridized to either the 3′ or 5′ probe are likely to contain a portion of a beta-tubulin sequence which carries an internal site for one of these restriction enzymes. The presence of 9–14 fragments which hybridize to the 3′ coding sequence probe suggests that the beta-tubulin gene number may be considerably larger than our minimum estimate. No differences in the patterns of hybridizing restriction fragments were detected between DNA samples from the cultivars 'Sun II' and 'Garry'.

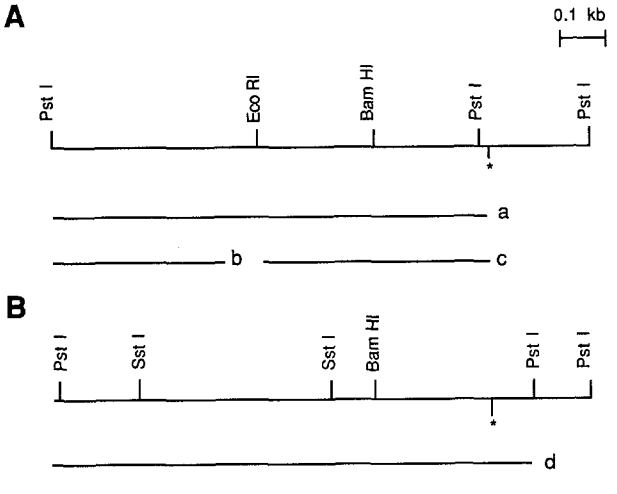

Fig. 1 A, B. Physical maps of oat beta-tubulin cDNA clones. The lines below the restriction maps represent the lengths and positions of the probes constructed from the $\beta 1$ (**A**) and the $\beta 3$ (**B**) cDNA clones, as described in Materials and methods. *Asterisks* indicate the positions of the stop codons

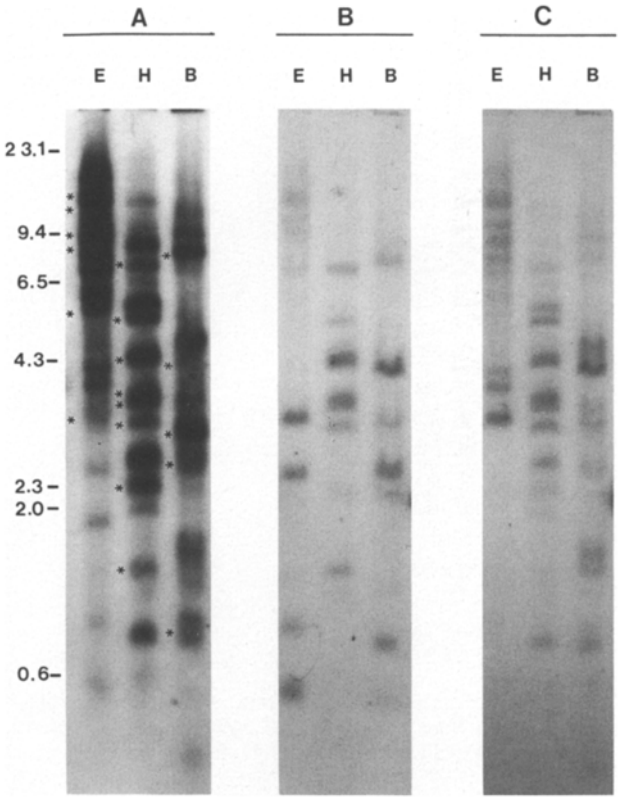

Fig. 2 A–C. Genomic-blot analysis of beta-tubulin genes in oat. Genomic DNA digested with *Eco*RI (*E*), *Hin*dIII (*H*), and *Bam*HI (*B*), gel-fractionated and blotted. The DNA was hybridized with the probes shown in Fig. 1. **A** Probes a and d, **B** probe b, **C** probe c. An autoradiograph of the blot is shown. Fragments hybridizing to both probes b and c are indicated by *asterisks* in **A**

Mapping of a beta-tubulin sequence to the chromosome missing in nullisome I

Genomic DNA isolated from disomic 'Sun II' plants and from 11 putatively different nullisomic plants was digested with *Hin*dIII, electrophoretically separated, and hybridized with probes a and d shown in Fig 1. A 2.3-kb *Hin*dIII fragment present in the disomic 'Sun II' DNA was absent in two different nullisomic I plants (Fig. 3) suggesting that the beta-tubulin sequence present on that 2.3-kb fragment was located on the chromosome missing in nullisomic I. This 2.3-kb fragment was present in DNA from all the other nullisomics (data not shown). The presence of the 2.3-kb *Hin*dIII fragment in DNA from two different monosomic I plants (Fig. 3) suggested that the absence

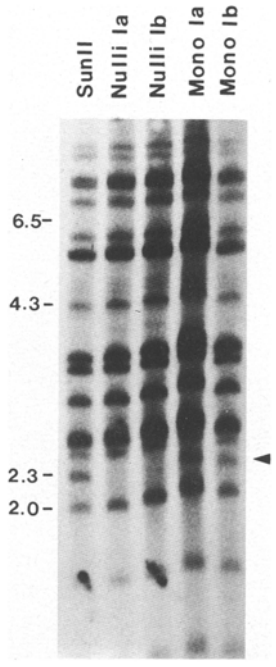

Fig. 3. Genomic-blot analysis of beta-tubulin genes in DNA from disomic 'Sun II', nullisomic I and monosomic I plants. DNA was digested with *Hin*dIII, gel-fractionated and hybridized with probes a and d (Fig. 1) A 2.3-kb fragment present in 'Sun II' DNA is absent in the DNA obtained from two different nullisomic I plants but present in the DNA isolated from two different monosomic I plants. All other fragments are present in all the other lanes

of the fragment in the nullisomic was not due to a restriction site polymorphism between the nullisomic and the disomic parent. These data supported our conclusion that the 2.3-kb fragment missing in nullisomic I maps to the chromosome missing in nullisomic I. The 2.3-kb *Hin*dIII fragment hybridized to both the 5′ and the 3′ non-overlapping ends of the cDNA (Fig. 2), indicating that it contained an entire beta-tubulin genomic sequence.

To obtain further confirmation of the absence of a beta-tubulin sequence in the nullisomic I line, DNA from disomic and nullisomic I plants was digested with either *Eco*RI or *Bam*HI and hybridized to the beta-tubulin probes. A 6-kb *Eco*RI fragment and a 4.3-kb *Bam*HI fragment were present in DNA from the disomic and monosomic I plants but absent in DNA from nullisomic I plants (data not shown). All other hybridizing fragments were similar among the different DNA samples. These data provided additional evidence that a beta-tubulin sequence (probably the same sequence revealed by three different restriction enzymes) maps to the chromosome that is missing in nullisomic I plants.

Localization of two beta-tubulin sequences to chromosome 2

A comparison of the *Hin*dIII restriction fragments which hybridized to beta-tubulin probes revealed two additional differences between the disomic 'Sun II' plants and the various nullisomic derivatives. Two fragments (4.3 and 3.5 kb) present in the DNA of the disomic plants were absent in DNA from several independent nullisomic VIII, IX and X plants yet were present in the DNA from the corresponding monosomic plants (Fig. 4). Both the 4.3-kb fragment and the 3.5-kb fragment hybridized to the 5′ and the 3′ non-overlapping probes, indicating that each of the two fragments contains a complete beta-tubulin coding sequence (Fig. 2). Because both fragments were absent in the same nullisomic lines, they must be located on the same chromosome. Similarly, a 3.5-kb *Bam*HI hybridizing fragment was present in DNA isolated from the disomic plant and from the three monosomic lines, but was absent in DNA from the three corresponding nullisomics (data not shown).

Of the three nullisomics VIII, IX and X, only nullisomic VIII has been characterized cytologically; it is deficient for chromosome 2, the largest satellited chromosome (Hacker and Riley 1965). One array of

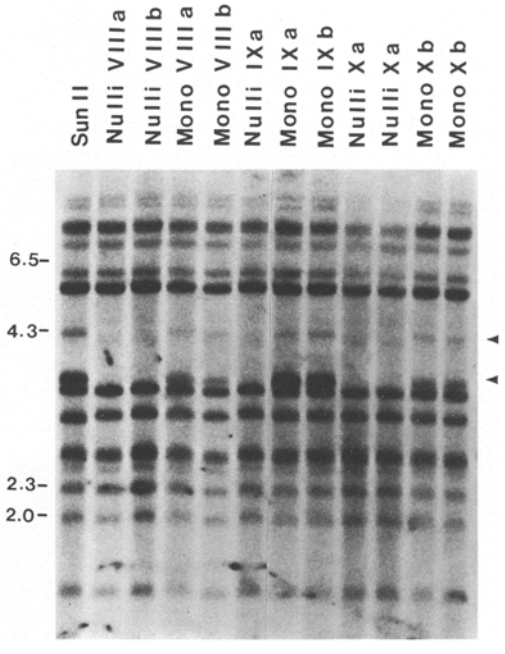

Fig. 4. Genomic-blot analysis of beta-tubulin genes in DNA from disomic 'Sun II' and nullisomic and monosomic VIII, IX and X plants. DNA was digested with *Hin*dIII, gel-fractionated and hybridized with probes a and d (Fig. 1). Two fragments of 4.3 kb and 3.5 kb, are present in 'Sun II' DNA and in the DNA of monosomics VIII, IX, and X but absent in the DNA from nullisomics VIII, IX, and X. All other hybridizing fragments are present in all the other lanes

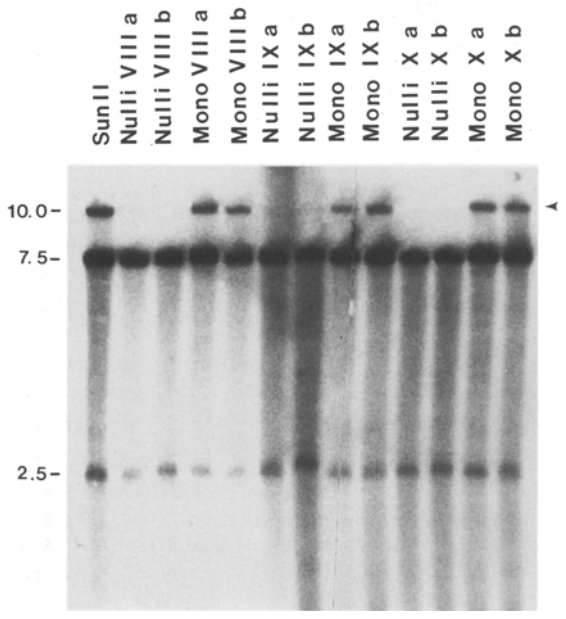

Fig. 5. Genomic-blot analysis of ribosomal RNA genes in DNA from disomic 'Sun II' and nullisomic and monosomic VIII, IX and X plants. DNA was digested with *Eco*RI, gel-fractionated and hybridized with the rDNA probe. The 7.5- and 2.5-kb fragments are present in all the lanes. The 10-kb fragment is present in 'Sun II' DNA and in the DNA of monosomics VIII, IX, and X but absent in the DNA from nullisomics VIII, IX and X

ribosomal genes, represented by a 10-kb *Eco*RI fragment containing an rDNA sequence, has been assigned to the satellite arm of chromosome 2 by a combination of Southern-blot analysis and in-situ hybridization to root-tip chromosome spreads (Jellen 1988). Because our beta-tubulin hybridization data suggested that nullisomics VIII, IX and X are all deficient for the same sequence, it was of interest to determine whether the nullisomic IX and X lines are also deficient for the rDNA array absent in nullisomic VIII. Hybridization of a maize rDNA probe to *Eco*RI-digested DNA revealed that DNA from nullisomics VIII, IX and X did not contain the 10-kb rDNA fragment present in DNA isolated from disomic 'Sun II', Monosomics VIII, IX and X (Fig. 5), and DNA from the other nullisomic lines (data not shown). These data are consistent with the placement of the two beta-tubulin genomic sequences, and an array of the ribosomal RNA genes, on satellite chromosome 2. Further, nullisomics VIII, IX and X are deficient in all, or at least a portion, of satellite chromosome 2.

Discussion

The results presented in this paper demonstrate the existence of a large family of sequences coding for beta-tubulin in cultivated oat. Copy number reconstruction experiments indicated that most of the hybridizing fragments contain two to five copies of tubulin sequences (data not shown). Because of the hexaploid nature of the genome, it might be expected that some tubulin genes on homoeologous chromosomes would be carried on restriction fragments of identical size. In this case, the hybridizing restriction fragment would not disappear in DNA from a nullisomic that lacked one of the homoeologous chromosomes. However, it is clear from our results that some of the hybridizing fragments correspond to a single sequence or a set of closely-linked sequences on a single chromosome because they disappeared in nullisomic strains.

Mapping of beta-tubulin sequences in oat demonstrates the value of DNA probes for dispersed, moderately repeated sequences in providing molecular markers for oat chromosomes. In this study, three different restriction fragments containing beta-tubulin sequences were mapped to two different chromosomes. The relatively small number of genes that were mapped can be attributed to the fact that the entire set of nullsomes representing all the 21 chromosomes was not available. The use of a complete nullisomic series should allow mapping of all the genes in a multigene family.

Cytological characterization of the monosomics and nullisomics used in this study has not been carried out partly because of the difficulty in identifying the different chromosomes. This study identifies potential problems with the existing aneuploid stocks, which have been described only on the basis of plant morphology and limited cytology, and in addition, demonstrates how molecular analyses can be used to characterize the available lines. Results from our hybridization experiments showed that nullisomics VIII, IX, and X are similar at the molecular level for the sequences we studied. However, plants from these lines had slightly different phenotypes. Nullisomic VIII plants were weaker than disomic plants, had smaller panicles with fairly large flowers, and were completely sterile and asynaptic. Nullisomic IX plants were extremely weak grass-like plants that produced comparatively large flowers, unlike nullisomic X plants, which often died before flowering (H. Thomas, personal communication). Two possible explanations could account for the observed molecular similarity and phenotypic dissimilarity of these lines. One possibility was that nullisomics VIII, IX and X are all nullisomic for all, or else a segment, of the same chromosome (chromosome 2) and exhibit different phenotypic characteristics because each possesses other unique genetic differences. These genetic differences could involve either simple variations or translocations, deletions, inversions and other chromosomal structural

rearrangements known to be common in hexaploid oat (Rajhathy and Thomas 1974). In this case, the monosomic lines should contain one copy of the diagnostic rDNA array and one copy of the two tubulin sequences, whereas nullisomic lines should contain no copies of these sequences. The second possibility was that nullisomic lines VIII, IX and X are each nullisomic for different chromosomes but, in addition, are homozygous deficient for either all or part of chromosome 2. In this case, both monosomic and nullisomic lines would be deficient in one or more of the diagnostic rDNA and tubulin fragments. Results from the hybridization experiments described above are consistent with the first model and suggest that the phenotypic differences observed among the three lines are not due to the absence of different chromosomes but are due to other genetic aberrations. Furthermore, N. Howes and coworkers at the Agricultural Research Cananda in Winnipeg, Manitoba, recently found that nullisomics of these three monosomic lines, VIII, IX, and X, also lack the same 26 kDa avenin polypeptide (personal communication). Thus the differences in phenotype and in the frequency of nullisomics described for these three classes (Hacker and Riley 1965; H. Thomas, personal communication) must be due to genetic differences in these lines other than the loss of either a large portion, or all, of a common chromosome.

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References

Cleveland DW, Sullivan KF (1985) Molecular biology and genetics of tubulin. Annu Rev Biochem 54:331–365

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation, version II. Plant Mol Biol Rep 1:19–21

Guiltinan MJ, Ma D-P, Barker RF, Bustos MM, Cyr RJ, Yadegiri R, Fosket DE (1987) The isolation, characterization and sequences of two divergent beta-tubulin genes from soybean (*Glycine max* L.). Plant Mol Biol 10:171–184

Hacker JB (1965) The inheritance of chromosome deficiency in *Avena sativa* monosomics. Can J Genet Cytol 7:316–327

Hacker JB, Riley R (1965) Morphological and cytological effects of chromosome deficiency in *Avena sativa*. Can J Genet Cytol 7:304–315

Hershey PH, Colbert JT, Lissemore JL, Barker RF, Quail PH (1984) Molecular cloning of cDNA for *Avena* phytochrome. Proc Natl Acad Sci USA 81:2332–2336

Hu N, Messing J (1982) The making of strand-specific M13 probes. Gene 17:271–277

Hussey PJ, Haas N, Hunsperger J, Larkin J, Snustad DP, Silflow CD (1990) Two β-tubulin genes are differentially expressed during development in *Zea mays*. Plant Mol Biol 15: 957–972

Jellen E (1988) Molecular genetics and restriction length polymorphism of ribosomal DNAs in oat (*Avena* species). *MS thesis*, University of Minnesota, USA

Marks MD, West J, Weeks DP (1987) The relatively large beta-tubulin gene family of *Arabidopsis* contains a member with an unusual transcribed 5′ noncoding sequence. Plant Mol Biol 10:91–104

McClure BA, Guilfoyle T (1987) Characterization of a small class of auxin-inducible soybean polyadenylated RNAs. Plant Mol Biol 9:611–623

Mendu N (1990) Oat tubulin genes and their expression during internode elongation. PhD Thesis, University of Minnesota, USA

Mendu N, Loer D, Colbert JT, Lissemore JL, Quail PH, Silflow CD (1985) Characterization of the tubulin cDNA clones of *Avena*. Proc 1st Int Congress of Plant Molecular Biology, p 86

Montoliu L, Rigau J, Puigdomènech P (1989) A tandem of α-tubulin genes preferentially expressed in radicular tissues from *Zea mays*. Plant Mol Biol 14:1–15

Mozer TJ (1980) Partial purification and characterization of mRNA for α-amylase from barley aleurone layers. Plant Physiol 65:834–837

Oppenheimer DG, Haas N, Silflow CD, Snustad DP (1988) The β-tubulin gene family of *Arabidopsis thaliana*: preferential accumulation of the β transcript in roots. Gene 63:87–102

Rajhathy T, Thomas H (1974) *Cytogenetics of oat (Avena L.)*. Genetics Society of Canada, Ottawa, Canada

Rigaud G, Grange T, Pictet R (1987) The use of NaOH as transfer solution of DNA onto nylon membranes decreases the hybridization efficiency. Nuleic Acids Res 15:857

Rines HW, Johnson SS (1988) Synaptic mutants in hexaploid oat (*Avena sativa* L.). Genome 30:1–7

Silflow CD, Oppenheimer DG, Kopczak SD, Ploense SE, Ludwig SR, Haas N, Snustad DP (1987) Plant tubulin genes: structure and differential expression during development. Dev Genet 8:435–460

Snustad DP, Haas NA, Kopczak SD, Silflow CD (1992) The small genome of *Arabidopsis thaliana* contains at least nine expressed beta-tubulin genes. Plant Cell 4:549–556

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517