Organisation and Expression of Plant B Chromosomes

by

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TABLE OF CONTENTS

CHAPTER 4: Transcriptional Analysis of Ribosomal RNA Internal Transcribed Spacer 2

CHAPTER 5: Cloning of the Ribosomal RNA Repeat Unit

ABSTRACT

Supernumerary B chromosomes are found in all major taxonomic groups of organisms. Although the B chromosomes significantly increase the nuclear DNA amount, no active genes have yet been unequivocally localised on these chromosomes by genetic or molecular means. However, the presence of B chromosomes is often associated with changes in chromosome behaviour, such as meiotic pairing and recombination, and it is therefore thought that they play a significant biological role. The mechanisms which are responsible for these effects remain to be determined.

Brachycome dichromosomatica is an Australian native ephemeral plant of the arid regions of South Eastern Australia. This species contains only two pairs of chromosomes in the normal complement (A chromosomes) and 0 - 4 B chromosomes in some populations. Sequences that hybridise to ribosomal RNA gene probes are present near the end of the short arm and, to a lesser extent, near the centromere of the B chromosomes of *B. dichromosomatica*.

In Chapter 3, internal transcribed spacer 2 (ITS2) was amplified by PCR from total *B. dichromosomatica* DNA using primers within the conserved regions encoding the 5.8S and 25S stable rRNA species. Comparison of the A chromosome ITS2 sequences, PCR amplified from several individual plants without B chromosomes, with corresponding sequences derived from microdissected B chromosomes revealed two consistent differences between the rDNA of A and B chromosomes. One of these differences produced an *Sfc* I restriction site that was present only in the ITS2 if the B chromosome rDNA. Amplification by PCR of ITS2 from total genomic DNA from plants with and without B chromosomes showed an additive relationship between the amount of PCR product containing the *Sfc* I site and the number of B chromosomes present. Quantitative

analysis indicated that the proportion of total nuclear rDNA present on a single B chromosome varied between 3 and 4% in plants.

In parallel experiments it was found that the rDNA of different chromosome races of *B. lineariloba* and *B. breviscapis* also contained the *Sfc* I restriction site but it was absent from *B. curvicarpa*, *B. dentata*, *B. eriogona*, *B. parvula*, *B. multifida*, *B. segmentosa* and *B. ciliaris*. Further sequence analysis of the ITS2 amplified from *B. lineariloba* (2n=16) identified an A at base 175, similar to that in the B chromosome ITS2, as well as an additional nine bases that differed from both A and B chromosome derived ITS2 sequences.

In Chapter 4, experiments using reverse transcriptase-PCR of the ITS2 region within the 40S precursor rRNA transcript suggested that the B chromosome rDNA was not transcribed in leaf tissue. Similarly, PCR of reverse-transcribed total leaf and flower RNA, using primers specific for the B chromosome ITS2, was unable to detect a transcript from the B chromosome rDNA.

In Chapter 5, molecular cloning of part of an A chromosome rDNA repeat unit enabled the complete sequence of the A chromosome intergenic spacer (IGS) region to be determined and revealed a putative RNA polymerase I transcription initiation site. In addition, three major regions of repeats, envisaged to have an important role in the regulation of rRNA transcription, were identified. Southern hybridisation with IGS-specific probes identified a *Xba* I restriction fragment polymorphism, present in genomic DNA containing B chromosomes and absent in genomic DNA without B chromosomes. This fragment is hypothesised to represent a B-specific IGS. Comparison of the PCR products amplified from genomic DNA with and without B chromosomes and from microdissected B chromosome DNA, with primers specific to the IGS region, identified several putative B

chromosome specific polymorphisms but no clear reason for rRNA gene inactivity on B chromosomes emerged from the sequence data.

In situ hybridisation with IGS-specific probes was not able to confirm the presence of polymorphisms in the region on the B chromosomes, but did corroborate the previous hybridisation of the heterologous cotton rDNA probe to the distal and proximal clusters of rDNA on the B chromosome, indicating that the *B. dichromosomatica* B chromosome contains major and minor loci for this sequence.

In Chapter 6, amplified fragment length polymorphism (AFLP) and random amplification of polymorphic DNA (RAPD) analyses were utilised as approaches to obtain low copy sequences specific to the B chromosomes or sequences common to the A and B chromosomes. AFLP analysis isolated two sequences (2aflp3 and 7aflp3) present in all B chromosome-containing samples examined. In addition, 2aflp3 was amplified from microdissected B chromosome DNA and from one sample without B chromosomes, suggesting that it was a sequence present on the B chromosomes and occasionally on the A chromosomes. 7aflp3 was not able to be amplified from microdissected B chromosome DNA and was postulated to have been isolated because of an A chromosome polymorphism.

RAPD analysis isolated a polymorphic sequence, BdER. Inconclusive evidence suggested that BdER was present on the A and B chromosomes and that it was not ubiquitous to either chromosome type within the population. Sequencing of BdER revealed significant nucleotide identity with retroviral and mitochondrial sequences, suggesting invasion of a subpopulation of A and possibly B chromosomes with mobile elements or organelle-derived DNA.