

PHYSICAL MAPPING of BRASSICA B-GENOME of *B. NIGRA* in CORRESPONDANCE to the FCA GENOMIC REGION of ARABIDOPSIS CHROMOSOME 4

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INTRODUCTION

The diploid B genome of Brassica species, although known to carry several important traits such as tolerance to various abiotic and biotic stresses, has not been studied as extensively as the related A and C genomes.

Physical mapping provides an accurate representation of a genome when the sequence is not available. A number of large insert libraries, the foundation for most physical maps, have been developed for Brassica species and have been exploited to facilitate map-based gene cloning for traits of interest and to provide insights into the evolutionary mechanisms that have formed these complex genomes.

OBJECTIVES

1. To investigate the physical organisation of regions of the B genome of *B. nigra* paralogous to a 222 Kb region of *A. thaliana* chromosome 4 that have been similarly characterized for both the A and C Brassica genomes.
2. To make comparisons between homologous regions in the paleo-ploid Brassica A, B and C genomes.

MATERIAL and METHODS

Construction of BAC library: The genomic DNA of *B. nigra* (DH No100) was used to construct a BAC library with an approximate 10 X genome coverage. The vector preparation, digestion, ligation and transformation was performed by BioS&T (<http://www.biost.com/>).

Screening and Sequencing: Eighteen genes from the *A. thaliana* FCA region of chromosome 4 (O'NEIL et al., 2000) were used to screen and identify homologous clones. BAC end sequencing was completed for 1,109 positive clones, these data were analysed to identify 500 non-redundant clones.

Southern Blot Analysis: BAC clones were purified and digested with *Hind*III enzyme and used in Southern blot hybridization to confirm the presence of the *A. thaliana* genes. The banding patterns were used to preliminarily assemble the contigs based on their shared bands (Fig. 1).

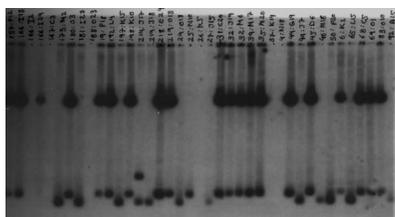


Figure 1. Southern analysis of selected BACs following *Hind*III digestion and hybridization with AT4g17460 gene as a probe.

SNaPshot Fingerprinting: BAC clones were purified and digested with 5 enzymes: *Eco*RI, *Bam*HI, *Xba*I, *Xho*I, *Hae*III. The 3' end was labelled with the SNaPshot reagent (ABI) and loaded on ABI 3130 for capillary electrophoresis (Fig. 2). Peak heights and sizes were collected by GeneMapper software and converted into FPC readable format using the GenoProfiler (LUO et al., 2003).

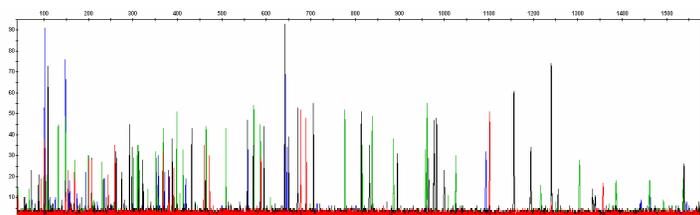


Figure 2. Electropherogram of a digested BAC using ABI3130 for fragment sizing. Restriction fragments in green are restricted with *Eco*RI, Blue with *Bam*HI, yellow with *Xba*I and red fragments with *Xho*I. *Hae*III produces blunt ends and hence not fluorescently labelled.

RESULTS and DISCUSSION

Twelve contigs has been assembled based on bands in common from 128 BACs which has been confirmed to carry more than one gene. Three of the finalized contigs are represented in Figure 3. Thus far, contig 3 shows the most similarity in comparison with its homologous region in the A and C genome (Fig. 4). However, the probing of the BACs is still in progress to finish the assembly of the contigs.

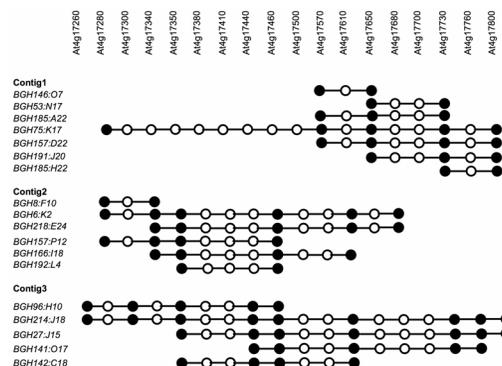


Figure 3. Three sample BAC clone contigs homologous to the Arabidopsis FCA region, assembled based on common hybridisation patterns. Closed circles indicate the gene specific clone hybridized to the BAC clone and open circles shows lack of hybridization of the gene.

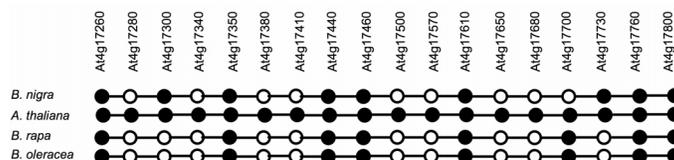


Figure 4. Comparison of the structure of the FCA region of *Arabidopsis* with the selected published contigs assembled for *B. rapa* and *B. oleracea* (PARK et al., 2005) and newly developed for *B. nigra*.

FUTURE INSIGHTS

The contig data is being refined and will be confirmed with the SNaPshot HICF fingerprinting method.

Selected BACs will be genetically mapped and fluorescent *in situ* hybridization will be used to locate them on B genome chromosomes.

A comparison between homologous regions in the paleo-polyloid Brassica A, B and C genomes will help uncover differential gene deletions and transpositions between the duplicated copies in the three genomes.

In addition, BACs representative of each region will be targeted for sequencing to elucidate the level of micro-scale rearrangements across the Brassica species divide.

REFERENCES

- LUO, M. C., et al., Genomics 82: 378-389.
O'NEILL, C. et al., Plant J 23: 233-243.
PARK, J. Y., et al., Mol Genet Genomics 274: 579-588.

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