SUMMARY
AND
CONCLUSIONS
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The present study was carried out to understand the genome organisation of *B. campestris* and its relationship with other allied genera and species belonging to Brassicaceae. Analysis of repeat DNA provides information about a large part of the genome. Study of single or low copy sequences provide information about regions which are responsible for important agronomic traits. Therefore, both repeat DNA sequences and single or low copy regions of the genome were studied.

1. One novel clone pBCMB69, 924 bases in length with 61.5% AT content belonging to a tandem repeat family was cloned and characterised in detail. This clone generated a ladder pattern with a basic monomer size of 550 bp. The repeat DNA clone has a limited distribution and hybridised strongly to few members belonging to Brassica lineage namely, *B. campestris*, *B. oleracea* and *B. napus* and faintly to *B. nigra* producing identical ladder pattern. Such a pattern of hybridization suggested a similar organisation in these genomes. Sequence analysis of the clone showed that the repeat DNA can be divided into two regions; a core region of about 570 bases and the flanking regions of about 200 bases on either side. The core region was unique in nature and showed no homology to any known plant DNA sequence. The flanking regions showed high levels of homology to 5S rDNA IGS and coding sequences from *Brassica* species. The presence of inverted repeats around the central core region indicates a possible role of transposition event in the evolution of the repeat family represented by the clone pBCMB69. Since the clone pBCMB69 exhibited a limited distribution, it was used to screen inter-generic and inter-specific hybrids in crop *Brassicas* having *B. campestris* as one of the parents.

2. One clone, pBCMB32 with 983 bases and 56% AT content belonging to a dispersed repeat family was isolated, cloned and characterized. The clone hybridized strongly to some members belonging to the Brassica lineage namely, *B. campestris*, *B. oleracea*, *B. napus* and *E. sativa* and faintly to members belonging to Sinapis lineage, namely *B. nigra*, *S. alba*, *D. siifolia*, *E. gallicum*, *M. arvensis* and *M. moricandioides*. The clone represents a class of transposons and retrotransposons as revealed by its homology to such sequences. Since the
clone was species-specific in nature, it was also used to screen intergeneric and interspecific hybrids between crop brassicas having \( B.\text{campestris} \) as one of the parents.

3. As a prerequisite for RFLP mapping, the inherent genetic variation between cultivars of \( B.\text{campestris} \) was assayed by RAPD. A total of 13 random primers used generated 135 amplification products. Of the 135 products, 110 were polymorphic. Accession specific products for YsPb, Tobin, Debra, Pantoria and Pusa kalyani were identified. The amplification products were scored as a binary matrix to calculate the similarity coefficient using Jaccard’s index. The similarity coefficient helped construct a dendrogram based on the UPGMA method. From the dendrogram it became clear that no two cultivars are identical and that the various cultivars are grouped into two clusters, A and B. All the yellow seeded, self-compatible cultivars were grouped in cluster A whereas all the brown seeded, self-incompatible cultivars were grouped in cluster B. The binary matrix was also employed to perform principal component analysis to generate a similar clustering of cultivars. Thus RAPD technique was able to produce unique fingerprint for each of the cultivars.

4. Based on the results on genetic variation among the different cultivars of \( B.\text{campestris} \), cultivars YsPb and Tobin from the two separate clusters A and B, respectively were selected as parents to generate the RFLP mapping population. YsPb was used as female and Tobin as the male parent to produce \( F_1 \) seed. A single \( F_1 \) plant was selfed to generate the \( F_2 \) seeds and the \( F_2 \) mapping population. A number of morphological and biochemical traits were scored. Statistical analysis indicated that the mapping population is highly variable and not homogenous. High values of standard deviation and coefficient of variation revealed that the population was influenced by environmental conditions and biased and therefore not suitable for RFLP mapping. Hybridization based RFLP was performed where only 3 out of 27 probes tested detected polymorphism. Therefore PCR based bulked segregant analysis (BSA) was carried out to tag genomic regions responsible for seed coat colour and erucic acid content.

5. BSA for seed coat colour loci was carried out with 158 random primers of...
which 9 detected 14 polymorphic products between the bulks varying in seed coat colour, yellow and brown. The linkage of these RAPD markers to seed colour was tested by co-segregation. For this, DNA from the two bulks along with the individual lines constituting the bulk were taken and amplified with the primer that had detected polymorphism during BSA. Only one primer, OPM-20 generated a product specific to bulk of yellow seeded plants (Y) and individuals constituting the bulk. This product termed as M-20_{11}, was found to co-segregate with the yellow seed coat trait. This can be used as a molecular tag for marker-assisted breeding using PCR approach.

6. BSA for erucic acid loci was carried out with 158 random primers, 16 primers generated 17 polymorphic products between the bulks of low èrucic acid lines and high erucic acid lines. To screen whether these polymorphic products were amplified in a phenotypic specific manner, DNA from the bulks and the individual lines constituting the bulk were amplified using the primer that had generated a polymorphic product during BSA. Only two primers, OPN-05 and OPK-01 generated the product in bulk and the individual lines in specific manner. The amplification product obtained with primers OPN-05 and OPK-01 were present in bulk of low erucic acid lines and the individual lines. These products, termed as N-05_{L10} and K-01_{L0.75} were found to be linked to erucic acid loci and thus can be used in marker assisted breeding using PCR technology. 

7. Finally, the fatty acid elongation 1 (Fae 1) gene, an important gene of the fatty acid biosynthetic pathway was cloned and characterized. The Fae 1 gene codes for β-ketoacyl co-A synthase of the elongase complex, responsible for elongation of C18 fatty acid moieties to C22 (erucic acid). This gene was cloned from 5 different sources namely F_{2} plants from B.campestris YsPb x Tobin cross containing low and high erucic acid, B.campestris cv. YsPb and cv. Tobin and B.oleracea. The clones from low erucic acid line (LEACAM Fae 1), high erucic acid line (HEACAM Fae 1) and B.oleracea (BO Fae 1) were sequenced and were found to be 1392, 1389 and 1380 nucleotides in length, respectively. These clones were deduced to translate for a protein that is between 458-459 amino acids in length. The clones showed high levels of homology to Fae 1 gene sequences from B.napus, B.juncea and A.thaliana. The clones shared high
degree of homology between themselves. The percent homology was 98.6% between LEACAM Fae1 and HEACAM Fae1, 98.2% between LEACAM and BO Fae1 and 98.8% between HEACAM Fae1 and BO Fae1. Thus, the Fae 1 gene from different species were near identical.

The repeat DNA sequences can be applied to understand phylogenetic relationships and to study evolution, to screen intergeneric and interspecific hybrids, DNA fingerprinting and cultivar identification. The RAPD markers developed through BSA can be used to monitor introgression of genes in other genetic background and as tags in marker-assisted breeding programmes.