#050

Specific whole-chromosome discrimination through oligonucleotide-based chromosome painting in Brassica species

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Background:

Brassica is a good model for studying polyploidization and for utilisation of interspecific hybridization for crop improvement, as the *Brassica* genus includes diploid species *B. rapa* (AA, 2n = 2x = 20), B. nigra (BB, 2n = 2x = 16), and *B. oleracea* (CC, 2n = 2x = 18), and their allotetraploid hybrids *B. juncea* (AABB, 2n = 4x = 36), *B. napus* (AACC, 2n = 4x = 38), and *B. carinata* (BBCC, 2n = 4x = 34). Interspecific hybrids between all these species can be produced, and show varying frequencies of non-homologous recombination between genomes which can be important for introgression breeding. Hence, identification of individual homologous and homoeologous chromosome pairs from different subgenomes in these species is important for plant breeding and evolutionary studies.

Objective:

The aim is to develop high-resolution oligo-based chromosome painting probes to identify subgenome-specific chromosome pairs. Establishing this method will allow high-precision analysis of chromosome rearrangements and introgression events in *Brassica*.

Methods:

Diploid A, B, and C genomes from *B. rapa*, *B. nigra*, *B. oleracea* and allopolyploid subgenomes AB, AC, and BC from *B. juncea*, *B. napus*, and *B. carinata* were used as input in the Chorus2 software for probe design. Short reads for repeat filtering from genome assemblies of B. rapa, B. nigra, B. oleracea, and B. napus were used. The design strategy used presence absence variants (PAVs) to identify unique genomic regions: oligonucleotide-based FISH-probes present only in one sub-genome (A, B or C). Chorus2 was run with all diploid genomes in one input file (A, B, C genomes), followed by filtering for repetitive sequences using short read data (k-mer-based using Chorus filter and select functions). Designed oligos were aligned against all diploid A, B, C genomes individually to check for haplotype-specificity and cross-hybridization to other genomes/species (AB, AC, BC genomes) (min. 60% identity over 60% length). Oligos specific to chromosomes *B. napus* A01 (An01) and *B. rapa* A01 (Ar01) and to *B. napus* C1 (BnC1) and *B. oleracea* C1 (Bo01) were selected for experimental analysis.

Results:

Oligo-based chromosome painting probes for chromosome A01 (B. rapa) and chromosome C1 (B. oleracea) were designed. A total of 20 000 oligo sequences designed for specificity along the length of chromosome A01 and 27 000 oligo sequences designed for specificity along the length of chromosome C1 were obtained. Probes showed high specificity to these chromosomes within allopolyploids containing the A and C genomes, with some minor exceptions where small regions with very low oligo-sequence density (< 25 oligos) showed in silico cross-hybridisation.

Conclusions:

We developed oligo-based chromosome painting probes specific to the entire A01 and C1 chromosome pairs. These oligo-based probes are able to discriminate in silico between these chromosome pairs in both *B. rapa* and *B. oleracea* genetic backgrounds and within the three different allotetraploid backgrounds of *B. juncea*, *B. napus*, and *B. carinata*. Cytogenetic analysis by fluorescence in situ hybridization using these oligo-based probes in mitotic chromosomes is underway in each of the Brassica species to confirm this specificity.