Molecular Cytogenetics in Onion Breeding.

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Abstract

The rapid progress in sequencing technologies and 'big data' analysis has opened boundless prospects for study of genome evolution and organization. However, we still have limited number of techniques allowing making a bridge between linear DNA sequence and genome structure. Molecular cytogenetics provides a toolbox of methods for DNA sequence visualization on nucleus or chromosomes bridging the gap between In Silico genome research and In Vivo genome organization. Molecular cytogenetic provides useful information for fundamental study of plant chromosome evolution and has practical application in breeding of new varieties.

From Sequences to Cytogenetic Markers

We employed the combination of sequencing, bioinformatic tools, and molecular cytogenetic methods for rapid development of cytogenetic markers for identification of individual chromosomes. Tandem repeats are often associated with important chromosomal landmarks, such as centromeres, telomeres, subtelomeric, and other heterochromatic regions, and can be good candidates for molecular cytogenetic markers. We have isolated several Tandem Repeat Sequences (TRs) from *Allium Cepa* and *A. Fistulosum* genomes which are valuable species specific cytogenetic markers associated with constitutive heterochromatin [1]. Knowledge of the chromosome organization of TRs may help to fill in sequence gaps that can arise during plant genome assembly, as TR arrays are difficult to sequence and assemble. Combining these results with CENH3 associated chromatin immune precipitation data we found that *Allium* centromere comprises the longest satellite monomer sequence known to date (~1250 bp).

Mapping Genes/Markers: Overcoming the Limitation of Detection Sensitivity with FISH

The ability to see a gene or marker on a physical chromosome has been the goal of many cytogeneticists. However, the FISH sensitivity for detection of single copy DNA sequences was limited, especially for highly compacted plant chromosomes. An ultra-sensitive method termed tyramide-FISH (tyr-FISH) was adapted for plant cytogenetics [2]. We used tyr-FISH to physically locate molecular markers tightly linked to the nuclear male-fertility (Ms) restoration locus of onion [3]. Relatively short genomic amplicons (846–2251 bp) and a cDNA clone (666 bp) were visualized on chromosome 2 near the centromere, a region of low recombination. This result explains why several labs have identified molecular markers tightly linked to the Ms locus after screening relatively few DNA clones or primers and segregating progenies.

Closely Related Wild species are actively used to improve the Germplasm of Cultivated Plants.

Gene collinearity is a prerequisite for the successful introduction of new traits because meiotic recombination occurs primarily within genes. Effective alien introgression strategies require high rates of homoeologous recombination between host and alien chromosomes. Using tyr-FISH we mapped members of the *Alliinase* multigene family on the physical chromosomes of two widely cultivated onion crops, *A. Cepa* and *A. Fistulosum*, unraveling unique genome re organization events [4]. Comparative target mapping of the *alliinase* genes and EST markers on the *A. Cepa* and *A. Fistulosum* mitotic chromosomes revealed a large ancient pericentric inversion involving the bulb *Alliinase* gene, the root (ALL1) *Alliinase* gene and the *Chalcone Synthase* gene (CHS-B).

Application of Molecular Cytogenetics in Interspecific Plant Breeding

Genomic In Situ Hybridization (GISH) has a huge advantage in its ability to distinguish between parental genomes in interspecific hybrids and thus allows the introgression of alien material to be monitored at the chromosome level. We used GISH in interspecific breeding of bulb onion (*Allium Cepa L.*) resistant to downy mildew (Peronospora destructor [Berk.] Casp.) [5]. Owing of GISH application the introgression lines that possessed an *A. Roylei* homozygous fragment in a pure A. cepa genetic background were produced. In interspecific breeding to monitor of the introgression of alien genetic material with molecular markers a huge number of markers should be produced in order to analyze the whole chromosomes of parental genomes. GISH allows easily selecting genotypes carrying the target locus without a wild-type background.

Another attractive aspect of the GISH is the ability to visualize the sites of recombination on the physical chromosome [6]. With GISH we studied advanced generations derived from hybrids between *A. Cepa* x *A. Fistulosum* that show resistance to *Stemphylium Vesicarium* **[7].** GISH showed spontaneous polyploidization and no evidence of backcrossing from interspecific hybrids between *A. Cepa* and *A. Fistulosum*. Recombinant chromosomes between *A. Cepa* and *A. Fistulosum* were identified revealing that introgression of disease resistances to bulb onion should be possible.

Future Directions

Future work involves the creation of more molecular cytogenetic markers based oSn mapping chromosome specific repeats and genes/markers with the subsequent integration of genetic and chromosomal maps. The resulting integrated maps can be further applied in onion breeding and genome assembly at chromosome level. Further analysis of the tandem repeats will clarify their role in speciation, heterochromatin formation, and function of a high packaged *Allium* chromosome. We expect a significant expansion of FISH applications in plants with developing of new technologies as oligo-FISH mapping, CAS-FISH for living cells, a haplotype-specific FISH for crossing over visualization, RNA-FISH for measuring gene transcription. In spite of the great progress in bioinformatic and genomic approaches, FISH remains a 'gold standard' method for mapping physical chromosomes and detection their organization.

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