P149 HIGHLY POLYMORPHIC SIMPLE SEQUENCE REPEATS (SSR'S) IN CHLOROPLAST GENOMES

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Simple sequence repeats (SSRs), consisting of tandemly repeated multiple copies of one- di- tri- or tetranucleotide motifs are ubiquitous in eukaryotic genomes, and are frequently used as genetic markers, taking advantage of their length polymorphism. We set out to examine the polymorphism of such sequences in organellar genomes of plants. GenBank searches identified presence of several single nucleotide stretches in plant chloroplast genomes. PCR amplification of a TnGm repeat adjacent to tRNAMet gene of soybean from several *Glycine max* cultivars, a *Glycine soja* line, and from several wild *Glycine* species revealed considerable repeat length polymorphism between wild *Glycine* species, but no polymorphism within *G.max*. The polymorphic amplified fragments were isolated and the DNA sequence was determined, confirming that the length polymorphism was caused by variation in the length of the repeated region. Amplification of a non-repetitive sequence adjacent to the T_nG_m shows no length polymorphism even between the wild *Glycine*. The chloroplast genome polymorphism described here can be used to follow the inheritance of the cytoplasm in genetic crosses using a simple PCR assay. A similar simple sequence repeat was identified by GenBank search in pine chloroplast genome (*Pinus contorta*, *Pinus sylvestris* and *Pinus thunbergii*), adjacent to a cluster of tRNA genes. DNA amplification of this repeated region from different pine species revealed several length variants, even among provenances within a single pine species. In the pines, the chloroplast genome is transmitted through pollen, and this PCR assay may be used for population genetic studies in *Pinus*. We expect that simple sequence repeats in the chloroplast genomes of other species will also be highly polymorphic. The finding described here implies that the slippage mechanisms assumed to play a role in the generation of simple sequence repeat polymorphisms in the nuclear genomes of eukaryotes also operate during the replication of the chloroplast genome.

P150 **CHROMOSOME-SPECIFIC LIBRARIES FROM FLOW-SORTED PLANT CHROMOSOMES.**

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This is an area of research that has received high propority in human genome project, but which has received only minimal attension in plants. Chromosome-specific DNA would have great value for many aspects of plant genome analysis. Flow karyotyping and sorting of chromosomes from tomato, petunia, rice, *Crepis capillaris*, corn and wheat will be described. Suspensions of metaphase chromosomes were prepared from cell suspension cultures or cultured leaf protoplasts or root tips. The isolated chromosomes were stained with fluorescent dye(s) and analyzed on a flow cytometer. Chromosomes from well resolved peaks on the flow karyotype were flow-sorted directly onto membrane filters for spot-blot hybridization analysis. The filters were processed and hybridized with chromosome-specific DNA probes in order to identify chromosome(s) corresponding to peaks in the flow karyotypes. Chromosomes were also flow-sorted directly into microfuge tubes. DNA specific to these chromosomes were amplified by using a novel PCR technique using universal 22-mer degenerate oligonucleotide primer (DOP) sequences. DOP-PCR yields a smear of fragments of various sizes from 250 to 1600 bp. Amplified products cloned into plasmid vector. Specificity of the clones to chromosome was confirmed by RFLP based linkage mapping. DOP-PCR products from sorted chromosomes are being used in chromosome painting studies by fluorescence *in situ* hybridization. The latest results on chromosome painting will be reported.

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PHYSICAL MAPPING OF SIX LEAF RUST RESISTANCE GENES INTROGRESSED INTO WHEAT FROM WILD RELATIVES.

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Fluorescence *in situ* hybridization was used as a diagnostic tool to detect physically the size of translocations for six different leaf rust resistance genes introgressed into cultivated wheat from different wild relatives. Detection of entire alien chromosomes or translocated arms has been routinely positive. Smaller alien introgression detection however, is more complex. We have successfully mapped the genes *Lr19*, *Lr24*, *Lr29* (*Thinopyrum elongatum*); *Lr25*, *Lr26* (*Secale cereale*) and *Lr37* (*Aegilops ventricosa*) on different chromosomes. The alien chromosome segments introgressed occupied approximately 75% (*Lr19*), 45%(*Lr24*), 40%(*Lr25*), 100%(*Lr26*), 63%(*Lr29*) and 29%(*Lr37*) of wheat chromosome arms 7DL, 3DL, 4AL, 1BS, 7DS and 2AS, respectively. Physical mapping of alien introgressions could be a faster means for tracking alien segments carrying the useful genes in a breeding population.

P152 **Generation of Expressed sequence Tags of *Brassica* plants.**

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Eight hundred fifty expressed sequence tags (EST's) of the root of cDNA clones of *Brassica napus* were generated by single-run partial sequencing. A computer search of these root EST's revealed that 122 EST's from 677 analyzed EST's show significant similarity to the protein-coding sequences in the existing databases including 22 clones related to the genes from other kingdoms. Northern blot analysis of the 10 database-matched cDNA clones revealed that many of the clones are expressed abundantly in root but less abundantly in other organs. However, two clones were highly root specific. The results show that generation of root EST's by partial sequencing of random cDNA clones along with the expression analysis is an efficient approach to isolate genes that are functional in plant root in a large scale. In addition, as an attemp to reveal the genes expressed in a specific plant cell type, we are generating ESTs from the cDNA clones of guard cells of *Brassica campestris ssp.chinesis*. The progress will be reproted.