# PLANT GENOMICS

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## Contents

1. Introduction to Genomics
2. History of Plant Genomics
3. Structure and Composition of Plant Genomes
4. Mapping Plant Genomes
4.1. Mapping Technologies
4.2. The Way Forward
4.3. Mapping Important Genes
4.4. Classic Quantitative Trait Locus (QTL) Analysis
4.5. Association Analysis and Germplasm Utilization
4.6. Positional Cloning: From Map Location to Gene
5. Functional Genomics
5.1. Northern Blot
5.2. Electronic Northern Blots
5.3. SAGE
5.4. Real Time PCR (rtPCR)
5.5. DNA Microarrays
5.6. Affymetrix Microarrays
5.7. Data Repositories
6. Small RNA Molecules
6.1. RNA Interference (RNAi)
6.2. Cross Protection
6.3. Virus Induced Gene Silencing
7. Conclusion: The Future of Plant Genomics
Glossary
Bibliography
Biographical Sketches



## Summary

Plant genomes dramatically vary in size and in function. In years past, genome size rather than economic or environmental utility determined which plant species were best characterized at the level of DNA sequence. Still, the broad outlines of plant genome organization and evolution were well-understood for most important crop species by the mid-1970's.

The diversity, both within and among species, that modern genetic tools reveal, is proving to be dazzling, and plant diversity is now being explored and utilized more effectively than ever before.

Plant biologists uncovered many of the most interesting facets of eukaryotic genomics, including transposition, retro-transposition and small RNA mediated gene regulation. New tools that are permitting the exploration, mapping and remodeling of plant genomes hold the promise to enable the consistent gains in plant productivity that our growing population and declining environmental resources will demand in the 21st century.

## 1. Introduction to Genomics

The focus of this chapter is on the plant nuclear genome. Plant chloroplasts and mitochondria maintain genomes, and plant mitochondrial genomes are large, variable and enormously interesting. Nuclear genomes of plants have been sculpted by inter-specific hybridization, polyploidy, transposition, retro-transposition, translocation and illegitimate recombination events. Wheat and maize provide excellent examples of several of these processes.

Durum wheat, used to make pasta, resulted from the hybridization of two diploid wheat grass species, *Triticum monococcum* and a grass very much like *Triticum speltoides*. The nuclear genomes of each of these related grasses contain fourteen chromosomes, and occasional failures of meiosis result in pollen and egg cells with fourteen, rather than the expected seven chromosomes. Durum wheat resulted from the fusion of two of these unreduced gametes, resulting in a plant with 28 chromosomes. Bread wheat resulted from the hybridization of an unreduced durum wheat egg by an unreduced pollen grain from the related grass, *Triticum tauschii*. Durum and bread wheat are examples of polyploids, organisms with multiple complete chromosome sets, derived from inter-specific hybridization events.

Maize achieves much of its remarkable diversity through the activity of transposons. These are DNA sequences that contain specific repeated sequences at their left and right boundaries that are recognized by enzymes that catalyze their movement from place to place in maize chromosomes. Transposons and retro-transposons help move genes and parts of genes throughout the genomes of higher plants, generating diversity, and also generate mutations by moving within or near genes. During meiosis, chromosomes occasionally mispair. When recombination occurs between mispaired chromosomes, part of one chromosome is swapped with part of another. One translocation differentiates the genomes of wheat and barley.

Gene duplication and divergence is common, and all of these genetic mechanisms result in what has been termed emergent complexity. We heterotrophs (organisms that derive nutrition by consuming other organisms) benefit from the variation and complexity that plant genome diversity provides. Human motivation for studying plant genomes is simple. Plants make our lives possible, and their beauty and utility are determined by the genetic programs stored within their genomes.

We all depend on plants for the air we breathe and the food we eat. As we move through the 21st century, our dependence on the plants that service our environment and provide food, fuel and fiber will become more evident. Our ability to utilize the genetic resources provided by evolution and to engineer novel solutions to current and coming

problems will be tested. Plant genomics is becoming an invaluable tool to those involved in crop improvement and germplasm management.

Plant nuclear genomes range in size from less than 100 million base pairs to more than 100 billion base pairs. Several explanations were offered to explain the apparent lack of correlation between genome size and plant form and function. The best current explanation for this variation is that replicative mobile genetic elements, primarily retro-transposons, invade eukaryotic chromosomes, and that genome size variation is primarily a consequence of different species' resistance to or tolerance of thousands of copies of these virus-like DNA sequences.

Genetics, bioinformatics, physiology and descriptive biology all contribute to genomics. Several plant genomes have been completely sequenced, but DNA sequence alone does not provide complete insight into the genomes' dynamic nature. We are now discovering that even among humans, substantial variation in the number of copies of genes can be observed among different individuals. Many of our most important crop plants (e.g. wheat and soybean) originated through inter-specific hybridization or whole genome duplication events, suggesting that we can expect plant genome architecture to be characterized by even greater variation both within and among species.

## 2. History of Plant Genomics

Formal research into the function of the plant genome started with Mendel in 1866. In a remarkable paper, Mendel demonstrated the particulate nature of genes, and provided proof of both segregation and independent assortment of genes. A surge of interest in genetics and its applications followed the confirmation, popularization and extension of Mendel's work (see also: *Plant Breeding and Genetics*).

In 1987 McClintock demonstrated the surprising capacity for specific maize genotypes to generate both forward and reverse mutations within several well-characterized genes at remarkably high rates. Her groundbreaking work with maize transposable elements led to an understanding of the phenomenology of transposition in both pro- and eukaryotes. Her insight into the adaptive role of transposition in the development of variation that contributes to local adaptation remains a central contribution to modern biology.

Several technologies ranging from quantitative Fuelgen stain analysis to DNA re-naturation analysis (see below) indicated that most crop genomes were large, and were full of repeated sequences. The activity of transposons and retro-transposons helped explain the initially confusing picture of higher plant genomes provided by DNA re-naturation analysis. These mobile genetic elements share several features with viruses, and have been termed genetic parasites. Still, they help generate the variation that natural selection acts upon, and in doing so contribute to plants' amazing capacity to adapt.

One of the mysteries of plant biology, the obvious improved performance of hybrid maize plants when contrasted with their parents, was initially correctly interpreted by Jones (1917), as a consequence of genetic linkage between undesirable recessive alleles

and desirable dominant alleles. Maize breeders exploit this feature of the maize genome by making crosses between appropriate parents, and producing hybrids that are homozygous for as few undesirable recessive alleles as possible. When Sax demonstrated in the 1920s that a gene impacting seed size in *Phaseolus vulgaris* was linked to a gene conferring seed pigmentation, scientists worldwide recognized the potential value of genome-wide linkage maps, which relate the relative positions of genes or markers on a chromosome based on the frequency of crossover between them during meiosis.

With enough genetic markers, one could utilize linkage analysis to identify all the chromosomal locations of the genes impacting plant performance, and could, theoretically, develop the 'best' variety for any environment. Elegant cytogenetic tools were developed to permit efficient chromosomal location of genes controlling Mendelian characters. By 1970, maize, wheat, pea and barley all had well-populated linkage maps. The typically recessive morphology-impacting genes that had been mapped were identified in specific genotypes, and compiling a sufficient number of these rare genetic variant alleles into a genetic tester line typically resulted in plants that performed poorly.

Botstein *et al.* (1980) pointed to DNA sequence polymorphism analysis as an approach that could, potentially, provide enough genetic markers for humans to permit genome-wide linkage map construction. Restriction fragment length polymorphisms (RFLPs) provided endogenous genetic markers sufficiently informative to make genome-wide linkage map construction feasible. For examples of the RFLP-based Southern Blots that permitted linkage map construction in barley, wheat and oat, go to http:\\wheat.pw.usda.gov. Linkage maps produced by RFLP analysis are also viewable on GrainGenes. Plant geneticists and plant breeders adopted RFLP analysis, resulting in the development of excellent, reasonably dense linkage maps in a wide array of plant species without the performance decay observed in plants carrying mutations that impacted plant phenotype (Tanksley and Fulton, 2006).

The development of polymerase chain reaction (PCR) sequence amplification coupled with increasingly inexpensive DNA sequencing technologies soon rendered RFLP mapping obsolete, and led to the development of remarkably efficient genotyping tools. Nonetheless, the RFLP maps that were generated in maize, tomato, wheat, barley and many other species permitted the chromosomal mapping of genes controlling economically important traits and led to the development of a genetic marker-assisted selection as a productive and practical plant breeding tool. Characterization of quantitative trait loci, genes impacting traits of interest in a quantitative rather than a qualitative Mendelian fashion, became a common practice in the 1990s. Several research groups were able to utilize genetic markers as starting points to identify and clone the genes impacting traits of interest, ranging from pathogen resistance to seed composition.

All plants derive from common ancestors, and structural similarities among genomes of relatively closely-related plant species are obvious. Among the *Triticeae*, seven pairs of mostly-metacentric chromosomes form the standard diploid genome. Barley and rye are diploids, with seven pairs of chromosomes. Durum wheat is a tetraploid, with fourteen

pairs of chromosomes and bread wheat is a hexaploid, with twenty one chromosome pairs. Synteny, the conserved physical relationship among genes on segments of chromosomes across related species, is generally observed across these species. When extended among to more distantly related rice and wheat or maize and rice, synteny can still be observed, although often gene locations have been altered by transposition or chromosome reorganization (Bennetzen *et al.*, 2005).

As DNA sequencing became less expensive and more efficient, sequencing genomes from a few plant species became an attractive and attainable goal. *Arabidopsis thaliana* was first sequenced; rice, grape and the model cool season grass *Brachypodium distachyon* soon followed. As the human genome program has demonstrated, within-species diversity is greater than we thought. Large-scale sequencing of the genomes of multiple individuals of important plant species will continue and will expand as sequencing costs decline. Although the cost of obtaining DNA sequence data continues to dramatically decline, assembling the relatively short DNA sequences in their proper order (this is called contig assembly) remains a daunting task. Since the genome of most crop plants contains transposons and retro-transposons that are imperfectly repeated thousands of times, it is difficult to know, when one sequences a retro-transposon, where in the genome it specifically belongs.

Several attempts have been made to estimate the number of genes expressed in the life of a plant. Estimates range from around 100,000 genes expressed during the lifespan of a tobacco plant to around 25,000 genes expressed in *Arabidopsis*. As the understanding of gene structure and regulation has advanced the interpretation of the plant transcriptome has evolved. Many research programs focused their effort on isolating messenger RNA from genes that were transcribed. These isolated mRNA molecules were copied to their complementary DNA molecules using the enzyme reverse transcriptase, and were then converted to double stranded DNA molecules which were then cloned and sequenced.

The relatively short (generally less than 1000 base pair) DNA sequences were called “expressed sequence tags” (ESTs) since the cloning process generally resulted in a truncated equivalent of the original mRNA molecule. EST cDNA sequencing projects have developed databases of sequences derived from isolated mRNA from many crops, with many plants providing hundreds of thousands of sequence files that have been condensed into contigs of related sequences. The barley (*Hordeum vulgare*) EST library contains more than 440,000 EST sequences that resolve into 28,000 contigs (http://harvest.ucr.edu/), each representing a gene or closely related family of genes.

Technologies permitting analysis of gene expression have advanced on pace with DNA sequence analysis techniques. While Northern blot analysis provided semi-quantitative analysis of gene expression, quantitative rt-PCR, microarrays, Serial Analysis of Gene Expression (SAGE), Illumina and Affymetrix technologies (see below) simultaneously permit analysis of most or all of the genes expressed in a tissue.

Among the most surprising discoveries in biology was the elucidation of small RNA mediated gene suppression, first discovered in plants (Herr and Baulcombe, 2004). In recent years, the RNA interference pathway has led to the development of novel

approaches to human disease management in addition to providing an entirely novel route by which we determine gene function.

As climate changes, the global population grows and competition for water resources increases, agricultural systems must become more productive and more efficient. Plant genetic resources provide still untapped reservoirs of genes that can contribute to better local adaptation and productivity. Plant genomics research provides insight into the genetic diversity that will be needed to prosper in the 21st century.

## 3. Structure and Composition of Plant Genomes

Plant genomes vary in genome size by a factor of 1000, but why they vary so dramatically is less obvious. Genome size variation appears to be poorly correlated with transcriptome complexity, and seems more dependent on the activity of transposable elements in the recent to distant past. Initially, researchers studying genome structure and composition relied on DNA re-naturation analysis. Typically, genomic DNA would be sheared to desired lengths by passage through a narrow orifice, appropriate size fractions would be denatured by heating and allowed to re-anneal under carefully controlled conditions. High copy number fragments would re-anneal more rapidly than would low copy number fragments, and single copy number fragments would find their complements and re-anneal last.

The rate of DNA re-annealing was monitored, and the relative proportions of DNA sequences of differing copy number determined. These analyses were represented as a $C_0t$ curve. As with most of the first generation biotechnologies, the reliability of results depended on the skill and insight of the scientists performing the assays.

## 4. Mapping Plant Genomes

### 4.1. Mapping Technologies

It was well-understood that genes could be organized into chromosome-length linkage groups through genetic linkage analysis if scorable allelic variants for those genes could be found. Prior to 1980, those alleles typically altered the plant phenotype in a way that could be scored visually. Genes with these 'naked eye polymorphism' alleles were localized to positions on chromosomes through the use of cytogenetic lines like trisomics or chromosome addition or substitution lines.

Prior to 1980 each of the chromosomes of barley, maize, wheat and *Arabidopsis thaliana* contained some genetic markers, but the amount of work required to map each gene and the scarcity of new 'naked eye polymorphisms' precluded the development of densely-populated linkage maps. Once restriction fragment length polymorphisms (RFLPs) were suggested as a route to develop dense linkage maps in humans, the route to reasonably dense, informative linkage maps in plants became clear. RFLP maps in maize, barley, rice, tomato, pepper and potato were produced in the 1980s.

RFLPs provide robust and reliable data that permit unambiguous interpretation of allele-state information for an unlimited number of genetic polymorphisms. Unfortunately,

this technology is dependent on highly skilled technologists, the use of large quantities of genomic DNA, and consumption of enormous quantities of nucleotide tri-phosphates labeled with radioactive phosphorous. Mullis' discovery of the DNA polymerase chain reaction (PCR) changed genotype analysis virtually overnight. With RFLP analysis, the electrophoretic mobility was determined of a fragment of DNA that comprises 1/1,000,000th of the DNA in the electrophoresis sample. This requires the exquisite sensitivity provided by the use of radioactively labeled DNA probes. PCR allows to amplify specific DNA fragments millions-fold, and directly determines their structural characteristics. PCR is less expensive than RFLP analysis, does not demand the use of radioactive isotopes, and demands far less technical skill than does RFLP analysis.

In the late 1980s and early 1990s, many research groups determined that a subset of genes contained repeat sequences composed of copies of dimeric (e.g. AC) or trimeric (e.g. CAG) repeats (Akkaya *et al.,* 1992). During DNA replication, these simple repeat sequences tend to change in copy number, and if one uses PCR primers that surround one of these variable repeat sequences, allelic variation can be observed as fragment length polymorphisms by gel electrophoresis. Genes that contain these simple sequence repeats (SSRs) have been found in all surveyed eukaryotes, and these have proven to be extraordinarily useful genetic markers.

DNA sequencing costs declined as sequencing technology improved. While it was impractical to consider sequencing most crop genomes in the 1990's, sequencing portions of large numbers of genes was straightforward. Messenger RNA (mRNA) is easy to isolate from plant tissues, and converting mRNA to DNA is easily done using reverse transcriptase and DNA polymerase. Once cloned, these DNA fragments are easily sequenced, and represent the sequences of the expressed genes from a tissue.

The Institute for Genome Research (TIGR) was the initial repository for these Expressed Sequence Tag (EST) sequences, and by 2003 more than 3 million plant EST sequences had been reported**.** These have been utilized to enable the development of gene expression tools and massively parallel single nucleotide polymorphism (SNP) genotyping technologies (http://www.illumina.com). Table 1 lists some of the world's plant genome databases.

| Name | Curators | Content |
|---|---|---|
| EMBL Nucleotide Sequence Database | http://www.ebi.ac.uk/embl/ European Bioinformatics Institute Hinxton, Cambridge, UK | Data and tools to analyze genes, gene expression, proteins, small molecules, pathways and much more. |
| The National Center for Biotechnology Information (NCBI) | www.ncbi.nlm.nih.gov National Library of Medicine, National Institutes of Health Beltsville, MD, USA | Genomes, nucleotide sequence information, pubmed, sequence analysis tools and more. |
| UK CropNet | ukcrop.net IGER, JIC, NASC and SCRI Six projects in four institutes in the United Kingdom | *Arabidopsis*, barley, Brassica, forage grasses, and millet databases and comparative analysis tools. |
| National BioResource Project (NBRP) | http://www.nbrp.jp A collaboration of many Japanese scientific institutes. | Resource center and repository for genomics projects. |

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| The IPK Crop EST Database (CR-EST) | ipk-gatersleben.de<br>Leibniz Institute of Plant Genetics and Crop Plant Research<br>Gatersleben, Germany | EST databases for wheat, potato, barley, pea, petunia and tobacco. |
| AFRICANCROPS.NET | www.africancrops.net<br>Alliance for a Green Revolution in Africa. Rockefeller Foundation, Bill and Melina Gates Foundation | African crop breeding networks, databases, seed source, news, free access portals, training programs. |
| J. Craig Venter Institute | www.jcvi.org<br>J. Craig Venter Institute<br>Rockville, MD, USA | *Brassicaceae*, *Medicago*, castor bean. rice genomics and much more. |
| Harvest | harvest.ucr.edu<br>University of California<br>Riverside, CA, USA | EST datasets and tools for barley, rice, citrus, cowpea, coffee, *Brachypodium*, wheat and soybean. |
| TAIR | www.arabidopsis.org<br>The Arabidopsis Information Resource<br>Carnegie Inst.<br>Stanford, CA, USA | Complete genome browser, extensive suite of tools and accession to genetic stocks. |
| OryzaBase | www.shigen.nig.ac.jp/rice/oryzabase<br>National Institute of Genetics in *Japan* | Integrated rice science, genetic map and mutant database |
| Gramene | http://www.gramene.org<br>USDA-ARS, Cornell University<br>Cold Spring Harbor, NY, USA | Comparative genomic analysis in the grasses with an emphasis on rice. |
| Graingenes | wheat.pw.usda.gov<br>USDA, ARS, Albany, CA<br>Cornell Univ., Ithaca, NY, USA | Genetic, genomic and phenotypic information for the Triticeae and *Avena*. |
| Plant GDB | plantgdb.org<br>Iowa State University<br>Ames, IA, USA | Maize EST database, tools and resources for plant genomics. |
| Panzea | panzea.org<br>USDA-ARS<br>Cornell University, Ithaca, NY, USA | Maize and teocinte genome data and software for genome and association analysis. |
| Soybase | soybase.org<br>USDA-ARS<br>Iowa State University, Ames, IA | Genetics data and tools for soybean and legumes. Breeders Toolbox. |
| CottonDB | cottondb.org<br>USDA-ARS<br>College Station, Texas, USA | Cotton Genome Browser, genes, genetics and taxonomy. |

Table 1. Some of the Many Plant Genome Databases containing plant genomics data.

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**Biographical Sketches**

**Thomas Blake** was raised on an olive orchard in Northern California. He received his B.S. in Genetics from the University of California (Davis) in 1976, his M.S. (agronomy) from South Dakota State University in 1979, and his Ph. D. from Washington State University (Genetics) studying under Dr. R.A. Nilan in 1983. He began mapping genes as a graduate student, and focused on mapping genes contributing to traits of agricultural importance when he joined the faculty of Montana State University as barley breeder and geneticist in 1984.

Dr. Blake took a 2-year leave from Montana State University to serve as Director of the Germplasm Program at the International Center for Agricultural Research in the Dry Areas (ICARDA) in 2002 and 2003. He returned to Montana State University in January, 2004, and is currently releasing drought tolerant 2-rowed malt and feed barley varieties.

**Victoria Carollo Blake** grew up in the San Francisco Bay Area. She received her B.S. in Biology from San Jose State University and her Ph.D. in Plant Biology from U.C. Davis. During her graduate studies in the Viticulture and Enology Department to characterize malic enzyme gene expression in ripening fruit, she would often spend fall semesters working the 'crush' in the Napa Valley for practical agricultural experience. She then took a Molecular Biologist position at the USDA ARS Western Regional Research Center in Albany, CA, to work as a curator for GrainGenes, the international database for the Triticeae and Avena.

In 2006, Victoria moved to Montana to join Tom Blake's lab and family in Bozeman. Her part of the barley project is to identify barley varieties whose forage and straw would provide a good feedstock for biofuel. They are making great progress in this area and hope to have a novel, inexpensive and environmentally friendly form of ethanol production from barley straw available by 2011.

**Jackie Campbell** received her B.S. degree (Biology) from the University of Colorado in 2004, and her M.S. degree from Montana State University in 2007, studying with Dr. Mike Giroux. Ms. Campbell has won several awards, including the Presidential Scholarship for Academic Excellence (University of Colorado), the Mildred Livingston Presidential Scholarship (Montana State University) and the Bayard Taylor Award for Outstanding Graduate Students (Montana State University).

Ms. Campbell is currently pursuing her Ph. D. (plant genetics) with Dr. Li Huang in the Plant Sciences and Plant Pathology Department, Montana State University. Her research topic is gene regulation, utilizing Barley Stripe Mosaic Virus as a vector for siRNA-mediated gene regulation.