

Sugarcane and Sugarbeet Biotechnology Report – Part I

The Plant & Animal Genomes XV Conference was held during January 2007 in its traditional annual meeting location of San Diego, CA. During the meeting, sugarbeet and sugarcane workshops were held. The sugarcane workshop has traditionally served as a meeting for the International Consortium on Sugarcane Biotechnology. Additionally, there were several technical presentations at the PAG meeting which dealt with sugarcane or sugarbeets. Abstracts of the sugarbeet presentations are presented here. Sugarcane abstracts will appear in a future issue of *Sugar Journal*.

Physical Mapping of Retrotransposons and Transposons In Beta vulgaris by High-Resolution Fish. **Thomas Schmidt , Beatrice Weber , Torsten Wenke , Daryna Dechyeva , Gerhard Menzel.**
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The genome of sugar beet has been investigated by fluorescent in situ hybridization to study the large-scale organization of transposable DNA sequences.

LTR retrotransposons show a contrasting chromosomal organization in Beta species. Ty3-gypsy-like retroelements, mostly rearranged, nested or truncated are amplified at centromeres. Many copies of the Ty3-gypsy retrotransposon Beetle1 (6738 bp) are inserted into Beetle2 (6684 bp). In contrast, Ty-copia retrotransposons show a genome-wide dispersion with depletion from centromeres and rRNA genes.

An ancient family of LINES is represented by BvL2 (6672 bp), containing two ORFs and a poly(A)-tail, and creating a target site duplication (TSD) of 18 bp upon integration. Conservation of insertion sites revealed transposition before radiation of Beta species.

Beta vulgaris SINEs are diverged into subfamilies with members sharing 18-94% similarity. SINEs are characterized by a poly(A) tail of 3-20 nucleotides and TSDs ranging in size from 3-19 bp.

The Mariner transposon Vulmar1 (3909 bp) contains a transposase ORF and is flanked by Terminal Inverted Repeats (TIR) of 32 bp. Using the TIR primer we have isolated three corresponding MITE classes showing that MITEs are indeed derived from Mariner transposons. Both Vulmar1 and VulMITEs are highly amplified and dispersed over all chromosomes including euchromatic regions. VulMITE I sequences are Stowaway-like elements and vary in size from 237 – 307 bp. By comparison of cultivated beet genotypes we have identified an active MITEs which has been mobilized in the last few hundred years.

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Rhizomania As Seen From Inside The Beet Cell: Identifying Proteome Differences Between Sugarbeet Infected With Beet Necrotic Yellow Vein Virus And Healthy Sugarbeet.

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Rhizomania, caused by Beet necrotic yellow vein virus (BNYVV) is one of the most economically important diseases affecting sugarbeet. The disease is characterized by excessive growth of lateral roots and constriction of the taproot, the main sucrose storage site in sugarbeet, resulting in decreased

sugar yield. The importance of this disease has been reemphasized by the emergence of new resistance breaking isolates in many areas where resistant sugarbeet is universally planted. This project focuses on identification of proteins induced or repressed during BNYVV infection, with a goal of determining key protein interactions between BNYVV and sugarbeet that contribute to disease. Near isogenic sugarbeet lines varying for the presence/absence of the Rz1 resistance allele were grown under identical environmental conditions in a growth chamber in noninfested soil or soil infested with BNYVV. At three and six weeks after planting, plant material was tested to confirm the presence/absence of BNYVV, and total plant protein was extracted from roots, quantified and fractionated using multidimensional liquid chromatography. Subtractive proteomics determined that only approximately 20% of the sugarbeet proteome was influenced during BNYVV infection compared with healthy sugarbeet. Protein identification using tandem MALDI-TOF-MS and sequence analysis has identified several major proteins influenced by infection that are known to be involved in cellular defense, including polyphenol oxidase, germin-like proteins, polyubiquitin and chitinase among others. Downstream analysis will involve arrays for the identification of interactions between BNYVV and sugarbeet proteins in an effort to identify key interactions driving infection and symptom development.

Molecular Genetic Tagging Of Beta vulgaris ssp. Maritima-Derived Resistance To The Sugar Beet Cyst Nematode, Heterodera schachtii.
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Resistance in commercial sugar beet hybrids to the sugar beet cyst nematode (SBCN) principally has been based on the Hs1 gene from the wild beet *Beta procumbens*, yet incorporation of this resistance has been detrimental to crop yield in nematode-free fields. Accessions of *B. vulgaris ssp maritima* were found to possess individuals exhibiting reduced cyst counts of the SBCN. These were used to produce segregating families of sugar beet by selection after recurrent back-crossing with a susceptible population C931. Individual plants from populations C927-4 and CN921-306, derived from different accession sources of *B. vulgaris ssp maritima*, were evaluated for reaction to SBCN in greenhouse assays. Data were used to group plants as either highly resistant or highly susceptible to SBCN. Random amplified polymorphic DNA (RAPD) analysis applied to the DNA samples from grouped plants revealed candidate amplicon markers associated with the nematode resistance. Successful conversion of RAPD markers to sequence tagged site (STS) markers permitted rapid marker evaluation of additional individuals from these populations as well as materials from a separate SBCN resistance-breeding program. The relationship between the markers obtained from the two different populations and our current understanding the *B. vulgaris ssp. maritima*-derived resistance to the SBCN will be discussed.

Molecular And Morphological Differentiation Among Sea, Ruderal and Cultivated Beets. **Piergiorgio Stevanato¹, Daniele Trebbi², J.**

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Knowledge of genetic diversity and relationships among *Beta vulgaris* genetic resources is essential for their conservation and development of breeding populations. In this study, we compared patterns of genetic variability and quantitative morphological data between a sea and a ruderal beet populations, collected on near sites of the Tyrrhenian coast of Italy, and a sugar beet variety. Eight amplified fragment length polymorphism (AFLP) primer-pairs were used for the genetic analysis whereas five traits of the root apparatus were evaluated for the morphological analysis in sixteen-day-old seedlings grown in hydroponics. Clustering analysis based on AFLP markers and root morphological data showed similar patterns of differentiation, in which sea, ruderal and cultivated beets appeared to be genetically distinct groups. A higher level of genetic variability was detected in the sea beet population, which may be due both to the limited gene flow between populations and the highly variable patterns of selection that occur in ecological niches, with respect than the genetic variability observed in the ruderal and cultivated beets. The results of this study revealed the extent of genetic diversity present within undomesticated beet populations that could be valuable in sugar beet improvement.

Evolutionary Conservation of the FLC Mediated Vernalization Response in Sugar Beet. **Christopher M. Richards¹, Partrick A. Reeves¹, Yuehui He², Robert J. Schmitz², Richard M. Amasino², Lee W. Panella³.** ¹USDA/ARS National Center for Genetic Resources Preservation Fort Collins, CO 80521 USA; ²Department of Biochemistry University of Wisconsin Madison, WI 53706 USA; ³USDA/ARS Northern Plains Sugar Beet Research Unit Fort Collins, CO 80521 USA

In many plants, exposure to a prolonged period of cold during the winter promotes flowering in the spring, a process termed vernalization. In *Arabidopsis thaliana*, the vernalization requirement of winter-annual ecotypes is caused by the MADS box gene FLOWERING LOCUS C (FLC). The extent to which this vernalization mechanism has been conserved during the diversification of flowering plants is not well understood. Using



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phylogenetic analysis, we identified homologs of FLC in species representing the three major eudicot lineages. FLC homologs have not previously been documented outside the family Brassicaceae. We show that the sugar beet (*Beta vulgaris*) FLC homolog (BvFL1), like FLC, is a repressor of flowering that is downregulated in response to cold. In addition, we show that allelic variants at this locus are more abundant in the wild progenitor subspecies *Beta vulgaris* subsp. *maritima* than in any of the cultivated lines we assayed. Flowering time is a trait of critical agronomic importance and considerable ecological interest. Assessment of functional variation at this key regulatory locus may be of value for ex situ conservation and may present an opportunity to study (and control) flowering time as a tool in applied plant breeding efforts.

Towards a Physical, BAC-Based Map of the Sugar Beet Genome.

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Sugar beet (*Beta vulgaris*) is an important crop plant in the European Union and in the United States. A physical map will constitute a powerful tool for marker assisted breeding, for straightforward positional cloning of genes and for

the integration of molecular resources that have already been generated for sugar beet. As part of the German Plant Genomics GABI-2 program, we are in the process of assembling a BAC map of the sugarbeet genome, integrated with both the sugarbeet genetic map, and the maps of the sequenced plant genomes of *Arabidopsis*, poplar, and rice. We have chosen a hybridization-based approach for generating the data set necessary for map construction.

12,000 probes will be anchored on the map, initially taken randomly, then by directed selection of probes, in order to close gaps between adjacent contigs and to maximize contig length and map continuity. We have presently compiled 31,488 sugar beet BAC end sequences, have generated pilot data for interspecific conservation and intraspecific divergence, and have generated 10,000 EST- and BAC end sequence-derived probes. Our project presently utilizes BAC resources representing 9-fold coverage of the sugar beet genome, and probe/clone relations for more than 7000 probes have already been determined. First-draft map assembly has been initiated. Our work will not only lead us to a global physical map, but will also constitute a first transcript map of the sugar beet genome, providing us with information on plant genome evolution, in terms of synteny. Concepts and status of the project will be presented.

GABI-Beet Physical Map: Generation Of A BAC-Based Physical Map Of The Sugar Beet (Beta vulgaris) Genome.

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Specific features of root enlargement and sugar deposition make sugar beet an interesting model for studying metabolism and development. Aim of the BPM project is the generation of a BAC-based genome map for sugar beet (*Beta vulgaris*) which is tightly linked to a genetic map with up to 2000 markers. The final map will be used for marker assisted breeding, positional cloning of genes of agronomic value, and for the integration of existing molecular resources.

The data necessary to establish the physical map will be generated by hybridization of site-specific 35mer oligonucleotides against high density arrays of BAC clones. The design of 35mer probes relies on existing sugar beet cDNA/EST data as well as BAC end sequences (BESs) that are generated as part of the project. The resulting contigs will be linked to the genetic map by genetic markers that target the same loci as a subset of the 35mer probes. PCR primers derived from the same sequence data (ESTs, BESs) are used to produce amplicons from two sugar beet genotypes that are parents of a mapping population. Sequence polymorphisms are detected by comparative sequencing. An important step in SNP detection is filtering out repetitive and duplicated sequences on the basis of a comprehensive analysis of repeat sequences. Finally, the SNPs are mapped using high-throughput MALDI-TOF-based SNP detection methods. We will report the status of this cooperative project of 4 academic and two industrial partners that is running since the beginning of 2005.