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Poster Abstract – D.01

CHARACTERIZATION OF EST-DERIVED SSR OBTAINED FROM A cDNA TOTIPOTENT LIBRARY OF DURUM WHEAT

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EST-SSR, wheat, microsatellites, molecular markers

Molecular markers are widely used in crop genetics and breeding. Genetic linkage, detection of quantitative trait loci (QTLs), positional cloning, and marker-assisted selection (MAS) are among the main applications. Genomic microsatellites have attracted relatively more attention because of their abundance in plants genome, reproducibility, high level of polymorphism, and codominant inheritance. Recently, due to the availability of enormous data for expressed sequence tags (ESTs), more emphasis has been given to EST-derived SSRs. They belong to the transcribed regions of DNA and have a higher rate of transferability across species than genomic SSR markers.

A collection of 10,000 ESTs deriving from a "totipotent" cDNA library constructed in durum wheat variety Ofanto (Patent n. WO2005003344) were screened for the presence of microsatellites.

One hundred forty out of these EST-SSRs were characterized in eleven durum wheat cultivars (Ciccio, Svevo, Latino, Primadur, Messapia, Creso, Pedroso, Ofanto, Cappelli, Orlù, Cosmodur) and in the *Triticum turgidum* var. *dicoccum* accession MG4343, parents of several mapping populations. Markers were opportunely chosen among di- and tri-nucleotide microsatellites in order to study relationships between number of repeat unit, type of motifs and level of markers polymorphisms. Of the 140 primer set tested, 85% amplified PCR products successfully and the other 15% failed to amplify any product. Multiple discrete PCR products were observed among both di- and trinucleotide EST-SSR markers including bands of expected and unexpected size. Differences between the expected and the observed size of the amplification products were observed. The data reported in the present work indicated the presence of a significant relationship between motif sequence types and polymorphism. The mean value of polymorphism was 35%. Polymorphism was calculated as number of primers that gave at least two alleles among the 12 genotypes analysed on the total number of primers amplifying PCR products. A set of Chinese Spring nullisomic and ditelosomic lines was also used in order to map markers on chromosomes.

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Poster Abstract – D.02

MOLECULAR EVOLUTIONARY AND PHYLOGENETIC IMPLICATIONS OF LINEAGE-SPECIFIC GROUP II INTRON GAINS AND LOSSES IN MITOCHONDRIA OF GYMNOSPERMS

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gymnosperms, mitochondria, group II introns, intron gains and losses, molecular phylogenetics

The rapid increase of complete mitochondrial (mt) genomic sequences in the public databases has revealed the uniqueness of their architecture, organization, expression and evolutionary history in plants. Huge size, additional genes, the presence of both Group I and Group II introns, the incorporation of foreign DNA from the chloroplast and the nucleus, high rate of recombination, and an ongoing functional gene transfer from the mitochondrion to the nucleus are typical features of the plant mt genomes.

Group II introns have been found in all land plants and algae mt genomes examined to date; their composition, position and distribution differs strikingly between vascular and non-vascular plant confirming the hypothesis that many independent intron gains and losses have occurred during evolution.

To get further insights into the pattern of group II introns allocation and conservation we came across the mt rps3 gene of a wide range of representatives of all the extant gymnosperms. According to a PCR assay and automated sequencing data, we now report the shared presence of two rps3 introns, namely the rps3i1 and the rps3i2 (Regina et al., 2005), in the mitochondria of the surveyed gymnosperms and unveil several remarkable exceptions among closely related species. On the whole our results provide compelling evidence that the distribution pattern of the rps3 introns is able to discriminate among divergent lineages of living gymnosperms and assist inferences of the evolutionary affiliations of particularly problematical representatives of this group of land plants.

Upon comparison of genomic and partial cDNA sequences, we also confirmed that both rps3i1 and the rps3i2 are accurately and efficiently excised in vivo. In addition, the comparative analysis of the novel rps3i2 orthologous sequences of Cycas, Ginkgo, Gnetum and Pinus allowed us to derive a secondary group IIA structural model.

These vascular plants constitute one of the major, but understudied plant group and occupy a pivotal position in evolution particularly with respect to the study of the seed plant origins and early divergence. Nonetheless, the relationships within the extant gymnosperm orders and families remain as yet questionable because of the diverse set of conflicting hypotheses generated in the past decade by morphological and molecular phylogenetic data.

To explore the phylogenetic utility of the mt intron sequences, we generated new mt molecular data sets combining sequence information from the rps3 locus and other mt sequences as well as information from all the three plant genomes of the same gymnosperm exemplar taxa. Our multigene and/or multigenome maximum parsimony and likelihood analyses support a phylogeny congruent with the conclusions reached by other molecular phylogenetic studies.

We propose, thus, the mt rps3i2 as a suitable informative character to highlight new mt genomic endeavours and diverse innovations characterizing the plant molecular biodiversity as well as to reinterpret the inter- and intrafamilial phylogenetic relationships among gymnosperms and to resolve the controversial position of Gnetales within seed plants.

Regina et al. (2005) J Mol Evol 60: 196-206

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Poster Abstract – D.03

A PRELIMINARY ANALYSIS OF *TdDRF1* GENE IN THE WILD WHEAT RELATIVES *TRITICUM URARTU* AND *AEGILOPS SPELTOIDES*

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(K.T. and E.T. contributed equally to this work. K.T. analyzed *Aegilops speltoides* genotypes; E.T. analyzed *Triticum urartu* genotypes)

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Crops are exposed to severe environmental stresses, such as drought that has detrimental effects on yields. Different genes have been described that are involved in the physiological and molecular responds to dehydration and low temperature resulting in plant adaptation. Breeding programs to improve the abiotic stress tolerance in wheat can benefit by a deep investigation on wild relatives that are a valuable source of genetic variation. Recently, *DREB* (Dehydration Responsive Binding factor) related genes, that play a key role in regulating plant abiotic stress response has been characterized in durum wheat and other cereals. In particular, the *DRF1* (Dehydration Responsive Factor 1) gene has been deeply characterized and analyzed in *Triticum turgidum* ssp *durum* (*TdDRF1*); it is about 3.4 Kb long, it codes for 2 transcription factors, belonging to the AP2/ERF family, and it is organized into four exons and three introns producing three different transcripts by alternative splicing. In particular, the exon 4 codifies for the AP2 DNA-Binding Domain and for the Nuclear Localization Sequence (NLS).

We report the identification of the *TdDRF1* homologous gene in the different genotypes of the wild progenitors *Triticum urartu* and *Aegilops speltoides*, donors of modern wheat A and B genomes, respectively. This gene in *T. urartu* and *A. speltoides* has been named *TuDRF1* and *AsDRF1*, respectively. Genotypes of *Triticum urartu* and *Aegilops speltoides* proceeding from geographically diverse regions have been selected for the analysis. A preliminary analysis of the *DRF1* gene sequence displays that the AA genomes (*T. urartu* accessions) are genetically closer between them and with respect to AA genome of the modern durum wheat than BB genomes (*A. speltoides* accessions). Furthermore, several SNPs, spread overall the gene sequence, including the well-conserved AP2 domain, have been found. These results could be useful for developing specific molecular markers related to the drought.On the other hand, these data coming from the screening of the wheat biodiversity, could be utilized for molecular taxonomy increasing the wheat genome knowledge.

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Poster Abstract – D.04

HEADING DATE QTL IN THE BARLEY 'NURE' X 'TREMOIS' MAPPING POPULATION

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barley, heading date, QTL

Temperate cereals can be categorized according to their response to prolonged periods of cold (vernalization - Vrn) and daylength (photoperiod - Ppd). Interactions between the two pathway result in a fine regulation of the transition from vegetative to reproductive growth. More than 80 Quantitative Trait Loci (QTLs) for heading date have been mapped up to now in different crosses in barley and they often concentrated in chromosome locations corresponding to previously known *Triticeae Vrn*, *Ppd* or '*Earliness per se*' genes. In the present work, 118 doubled haploids lines deriving from the 'Nure' x 'Tremois' cross were genotyped with the DArT technique to create a medium density linkage map (more than 500 loci). The same NT population was grown in different, controlled conditions (phytotron) of vernalization and photoperiod. QTL analysis confirmed that the 'Nure' x 'Tremois' genetic system supports the *VRN-H1/VRN-H2* two locus epistatic model for vernalization requirement previously proposed, based on phenotypic segregation, mapping data, and allele sequence at candidate loci. Besides *VRN-H1* (chromosome 5H) and *VRN-H2* (chromosome 4H), other genomic regions controlling the days to heading trait were identified on chromosomes 1H and 2H. Interestingly, the *HvBM8* gene, encoding for a MADS-box transcription factor, was mapped in coincidence with a highly significant QTL determining heading date on chromosome 2H. Functional characterization of *HvBM8* is being carried out in order to confirm its involvement in the control of barley vegetative to reproductive transition.

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Poster Abstract – D.05

FLUORESCENT BAC-FINGERPRINTING TECHNOLOGY TO CONSTRUCT CHROMOSOME-SPECIFIC PHYSICAL MAPS OF WHEAT

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Triticum aestivum, fluorescent fingerprinting, physical map

Triticeae are the main cereal crops in Europe. For many years the size and complexity of the wheat, barley and rye genomes have hampered the development of genomics and its application to produce *Triticeae* crops with improved composition and characteristics. Recently, however, new and more efficient scientific capabilities and resources have been developed that allows robust genomic programs to be established for the *Triticeae*.

The EU-FP7 project *TriticeaeGenome* is designed to achieve significant progresses in *Triticeae* genomics and support efficient breeding of improved varieties for the European agriculture.

In the framework of this project, the Institute of Applied Genomics (IGA), is committed to build physical maps of three chromosomes of *Triticum aestivum* (1A, 1B, 3D), starting from chromosome-arm specific BAC libraries built from Dr Dolezel group in Olomouc (Czech Republic) and using a modified version of the High Informative Content Fingerprinting Technology developed by Luo *et al.* (Genomics 2003). In particular six chromosome-arm specific BAC libraries will be fingerprinted for a total number of 400,000 clones correspondent to a 15x coverage per chromosome.

BAC DNAs will be isolated, simultaneously digested with 5 restriction endonucleases, and electrophoresed on a capillary automated sequencer after 4 fluorescent dye labeling. In order to reduce costs and increase throughput, all the phases have been modified with respect to the protocol already utilized at IGA to construct other physical maps (i.e. grapevine).

Here these modifications to the fluorescent BAC-fingerprinting procedure will be presented.

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Poster Abstract – D.06

ISOLATION, FUNCTIONAL CHARACTERIZATION AND MAPPING OF A *P*-COUMAROYL ESTER 3´-HYDROXYLASE GENE (C3'H) IN GLOBE ARTICHOKE

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Cynara cardunculus var. scolymus, C3'H, phenylpropanoid metabolism, genetic mapping

Globe artichoke (*Cynara cardunculus* L. var. *scolymus*) is a perennial and cross pollinated vegetable, native to the Mediterranean area, which is usually vegetatively propagated. The major use of globe artichoke is for human food, but various studies have demonstrated health-promoting properties of its leaf extracts, mainly due to their polyphenolic content. The dominating phenolics are di-caffeoylquinic acids and chlorogenic acid (5-caffeoylquinic acid).

The metabolism of dicaffeoylquinic acid in plant is still unknown, and the definition of chlorogenic acid synthesis remains controversial. Both chlorogenic and dicaffeoylquinic acids derive from the phenylpropanoid pathway and contain an -OH at '*meta*' position on aromatic group. The CYP98 cytochrome P450 monoxigenase gene family is the most likely candidate class of enzymes catalyzing the 3´-hydroxylation of phenolic compounds.

By applying a CODEHOP strategy, we isolated and characterized the full-length cDNA of the globe artichoke C3´H (*p*-coumaroyl ester 3´-hydroxylase) gene, involved in both chlorogenic acid and lignin syntheses. Phylogenetic analyses demonstrated that this gene belongs to the CYP98 family.

Functional analysis was perfomed by expressing the C3´H gene in yeast with an *Arabidopsis thaliana* cytochrome P450 reductase *ATR*. The gene was found very active in converting the *p*-coumaroylshikimate into caffeoylshikimate. In contrast, the conversion of *p*-coumaroylquinate in caffeoylquinate was very slow and only detectable at a high concentration of substrate (100 μM).

The C3´H promoter sequence was analyzed for identifying potential regulatory elements; a putative TATA box was found 33 bp upstream of the transcription start site and a putative CAAT box at 145 bp upstream. Real Time PCR analysis demonstrated higher expression level of C3'H following exposure to UVC, which we found to induce increases in dicaffeoylquinic acids biosynthesis.

We analyzed the allelic forms of C3´H gene in two globe artichoke genotypes: 'Romanesco C3' (a late maturing, non-spiny type) and 'Spinoso di Palermo' (an early maturing spiny type), which are the parents of an F_1 segregating populations we previously used for the development of the first genetic linkage map, based on a two way pseudo-testcross strategy. A SNP was identified making possible to localize the C3'H gene on the linkage group 10 of both maps, thus increasing the number of the shared intercross markers; The gene is located at about 2 and 7cM respectively from the microsatellite CELMS-39 and the AFLP markers P45/M47-06.

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Poster Abstract – D.07

EST-SSR MARKERS DEVELOPMENT FOR MAPPING AND PHYLOGENY STUDIES IN EGGPLANT

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Solanum melongena, simple sequence repeats, linkage mapping, BLAST analysis

Eggplant (*Solanum melongena* L.) is a member of the *Solanaceae* family, but unlike most of the solanaceous crop species, it is endemic to the Old, not the New World. In spite of its widespread cultivation, and its nutritional and economic importance, its genome has not as yet been extensively investigated. Few analyses have been carried out to determine the genetic diversity of eggplant at the DNA level, and linkage relationships have not yet been well characterised. As for the other *Solanaceae* crop species (potato, tomato and pepper), the level of intra-specific polymorphism appears to be rather limited, thus it is important that an effort is made to develop more informative DNA markers to make progress in understanding the genetics of eggplant and to advance its breeding. The aim of the present work was to develop and characterize a set of functional microsatellite (SSR) markers via an *in silico* analysis of publicly available DNA sequences, and to evaluate their transferability among other solanaceous species.

A computer search of 3,357 sequences from the *Solanaceae* Genomics Network database (SGN; http://www.sgn.cornell.edu) and EMBL Nucleotide Sequence Database (http://www.ebi.ac.uk/embl), was performed using the Sequence Retrieval System (SRS6, http://srs.ebi.ac.uk/). In order to obtain non-redundant sequences containing SSRs, a cluster analysis was performed; the contigs obtained were carefully evaluated and redundancies were removed. The non-redundant sequence pool contained 1,864 sequences corresponding to approximately 740 Kb. Within these, 64 sequences contained one or more SSRs (including 20 mono-, 11 di-, 36 tri-, one tetra- and two hexanucleotide motifs). Primer pairs could be designed for 50 SSR-containing sequences, the remaining contained either too little DNA sequence flanking the microsatellite or the sequences were inappropriate for primer modelling. Thirty-nine EST-SSR were functional, and were then applied to a panel of 44 accessions, made up from 38 cultivated eggplant varieties, breeding lines and rootstocks, and six related wild *Solanum* species: *S. viarum, S. sodomaeum, S. sisymbrifolium, S. torvum, S. aethiopicum* and *S. integrifolium*

The usefulness of the SSR assays for diversity analysis and taxonomic discrimination was demonstrated by constructing a phylogeny based on SSR polymorphisms, which was in good agreement with prior taxonomic classification based on both genomic and plastidial markers. Most EST-SSRs were also functional when tested with templates from tomato, pepper and potato. As a results of BLASTN analyses, several eggplant SSRs were found to have homologous counterparts in the phylogenetically related species, which carry microsatellite motifs in the same position.

Since EST-SSRs lie within expressed sequences, they have the potential to serve as perfect markers for genes determining variation in phenotype. Their high level of transferability to other *Solanaceae* species can be used to provide anchoring points for the integration of genetic maps across species.

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Poster Abstract – D.08

MOLECULAR ANALYSIS OF A SUNFLOWER GENE ENCODING A HOMOLOGUE OF THE B SUBUNIT OF A CCAAT-BINDING FACTOR

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gene expression, Helianthus annuus, transcription factors, methylation, promoter region

The *Helianthus annuus LEAFY COTYLEDON1-LIKE* (*HaL1L*) gene encodes an NF-YB (or HAP3) of a CCAAT box-binding factor (NF-Y). The peptide HaL1L results homologous of the LEC1-LIKE of *A. thaliana*, sharing a high amino acid sequence identity (56%). *HaL1L* transcripts are accumulated primarily at an early stage of sunflower embryogenesis. High levels of *HaL1L* mRNA have been detected in the developing embryo proper, suspensor, endosperm, integument, and integumentary tapetum cells, while no or low transcript levels were detectable in organs such as the cotyledons, leaves, stem internodes, roots, and unfertilized ovules (Fambrini et al., 2006 Dev Genes Evol 216: 253-264). A large insert genomic library from *H. annuus* was successfully screened to isolate the entire *HaL1L* gene. From GenBank databases analyses it has been suggested that the identified genomic DNA fragment is homologous to the *A. thaliana* chromosome V region carrying *AtL1L* and the immediately adjacent genes at the 5' and 3' sides respectively. In the *HaL1L* 5' flanking region, elements peculiar to a putative TATA-box promoter and two "CG isles" were identified. An investigation on the methylation status of the CG rich DNA regions shows that differentially methylated cytosines are recognizable in DNA of embryos at the fifth day from pollination (DAP) in comparison to the leaf DNA. These data suggest an epigenetic regulation of *HaL1L* transcription carried out by methylation of cytosine residues during plant development. The observation that *HaL1L* mRNA is downregulated in leaf tissues and reach the higher steady state level in 5-DAP embryos support the results of methylation analyses (Fambrini et al., 2006). The nucleotide sequences were also analyzed to individuate *cis*-regulatory sequences involved in the *HaL1L* transcription regulation by other transcription factors (Yamamoto et al., 2007 BMC Genomics, 8: 67-90). One of the most intriguing motifs, present in the 5' flanking region as well in the *HaL1L* intron, is WUSATAg. It represent the target sequence for the transcription factor WUSCHEL (WUS) (Mayer et al., 1998 Cell, 95: 805-815), which could by involved in the complex regulation system controlling the zygotic embryo development. As regard to the 3' region, in addition to the nuclear polyadenylation signal, a cytoplasmic polyadenylation signal which suggest a negative post-transcriptional regulation was also identified. Poly(A) tails, lengthened by cytoplasmatic poly(A)polymerases (PAPs), form complexes with regulative proteins which inactivate mRNAs. During embryo development PAPs act under hormonal control (Rothnie, 1996 Plant Mol Biol, 32: 43-61). Noteworthy, the presence of *ARF* and *ABRE* motifs in the *HaL1L* promoter region suggests auxin and abscisic acid involvement in the expression control of this gene. The hypothesis of a translational control for *HaL1L* is also supported by the *in situ*

hybridization analysis (Fambrini et al., 2006), that demonstrate an accumulation of *HaL1L* transcripts in maternal tissues of developing embryos such as integument and integumentary tapetum cells. On the basis of our study a control of *HaL1L* expression mediated at transcriptional level by both methylation of cytosine residues and interaction with other transcription factors is suggested. In addition, a control at translational level by a temporary unavailability of pre-synthesized *HaL1L* mRNA could be also supposed.

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Poster Abstract – D.09

CHARACTERIZATION OF FLC-LIKE SEQUENCES IN *C. INTYBUS*

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flowering transition, vernalization, inflorescence, FLC, chicory

In winter annuals ecotypes of *Arabidopsis*, the flowering repressor, *FLOWERING LOCUS C* (*FLC*), a MADS box transcription factor, is expressed at such level as to inhibit flowering in the first growing season. FLC expression is enhanced by *FRIGIDA* (*FRI*) to levels that inhibits the transition to flowering by repressing the expression of the genes often referred to as Floral Pathways Integrators. The main process promoting flowering by the repression of *FLC* is the vernalization and the duration of cold has been shown to be proportional to the degree of down-regulation of *FLC*; such repression is maintained for the rest of the plant life even after cold exposure ends, but is restored after meiosis. The repression involves epigenetically stable modifications in *FLC* chromatin that include a H3 Lys27 trymethylation (H3K27me3) and a H3 Lys9 methylation, (Sung et al, 2006).

Wild chicory (*Cichorium intybus* L.) is a biennial species which requires vernalization to flower. In Italy different types of chicory (the so called Italian red and variegate types) have been selected by farmers as leafy vegetable. These types show quite different classes of precocity in relation to flowering.

In our study, we are investigating the molecular basis that regulate the switch to flower in chicory by vernalization, to verify whether such mechanism is the same that controls flowering in *Arabidopsis*, and, finally, to address the diversity of the classes of precocity to one of the cases known for this model plant. We isolated FLC homologues from chicory and characterized their expression in plant tissues and we studied the pattern of cytosine methylation in chicory genomic DNA in response to vernalization. Given the presence of 4 *Ci*FLC transcript variants in chicory, we tested by Southern blot analysis the number of FLC copies in the *C. intybus* genome of different cultivar of chicory. Souther analysis and GenomeWalking led to the isolation of five sequences: two of them corresponding to intron-less cDNA-like sequences where the start codon ATG was replaced by a TGA stop codon; a third sequence, 439 bp in length, corresponded to the "putative" second exon and the beginning of the second intron of the gene; a fourth genomic sequence of 283 bp, is very peculiar, because it presents a partial duplication of the MADS-domain and the two repetitions are separated by a non-coding 85 bp region. A second southern blot has cleared the presence of multiple copies of the *CiFLC* gene, using as a probe a DNA sequence of 439 bp isolated by genome walking.

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Poster Abstract – D.10

ANALYSIS OF CLONAL VARIATION IN GRAPE CULTIVARS

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grape clones, somatic variation, re-sequencing, next generation (re)sequencing, comparative genome hybridization

Grapevine is a vegetatively propagated fruit crop. Many grape varieties propagated by grafting since centuries undergo natural mutations (base mutations, indels, transposable element insertions or excisions, and epigenetic modifications) that can lead to the appearance of desirable phenotypic variations. These genetic variants are selected and commercialized as a new clone of a certain cultivar by nurseries, but up to now there is no reliable tool to discriminate clones from one another and clones can not be patented and protected from illegal multiplication.

The grape material that IGA is analyzing is composed of 3 pairs of clones belonging to 3 different varieties plus 4 clones for the Tocai variety: (1) Pinot noir VCR18 clone vs Pinot Blanc VCR5; (2) Sauvignon R3 clone vs Sauvignon french clone 297; (3) Sangiovese VCR5 clone, biotipo toscano vs Sangiovese VCR23 clone, biotipo romagnolo; (4) Tocai R5 clone, Tocai R14 clone , Tocai French clone and Sauvignonasse.

In the starting phase of the project, eight clones (the first 3 pairs and two Tocai clones, R5 and R14) were genotyped at 180 microsatellite loci scattered across all 19 chromosomes starting from DNA extracted from apical leaves and berry skins. The AFLP (Amplified Fragment Length Polymorphisms) technique was used in parallel to SSR analysis to discover single base polymorphisms due to mismatches or small indels. The two approaches with standard molecular markers provided a reduced amount of information about clonal variation. Then a re-sequencing effort using standard Sanger sequencing has been initiated for Pinot blanc cultivar. More than 41.000 mate-paired reads were produced, correspondent to about 28 Mbp of Pinot blanc genome (0,06x coverage). Mate-paired reads are currently being aligned to the PN40024 genome (Nature, 27 August 2007) and to the Pinot noir ENTAV genome (PloS One, 19 December 2007) to detect the presence of nucleotide and structural variation between Pinot clones.

With the availability of next generation sequencing technologies and the accessibility at low costs of other whole genome scale approaches (i.e. whole-genome scanning microarrays), IGA has started the analysis of grape clones genomes by two approaches: (1) Ultra high-throughput sequencing of reduced representation libraries by the Illumina Genome Analyzer GAII; (2) Microarray based Comparative Genomic Hybridization (CGH) to detect insertion/deletions and DNA copy number differences between couple of clones belonging to the same variety.

The results of such genome wide analyses of grape clones will be transferred to a high throughput, highly automated protocol for the routine analysis of clonal variants

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Poster Abstract – D.11

RESEQUENCING OF A SECOND GRAPEVINE GENOME REVEALS FREQUENT SNP AND STRUCTURAL VARIATION

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re-sequencing, grapevine, genome, nucleotide variation, structural variation

The grapevine (*Vitis vinifera*) is economically the principal fruit plant in the world. Using a highly homozygous genotype, PN40024, derived from selfing of Pinot Noir a high quality draft sequence assembly was produced at 12X coverage with a whole genome shotgun approach. 90% of the 12X genome sequence assembly that totals approximately 485 Mbp is anchored in 192 pieces on the 19 chromosomes.

The re-sequencing of a second genotype, the Italian cultivar Tocai friulano, allowed the identification of single nucleotide polymorphisms and structural variations (SVs) between the two genotypes. This analysis was achieved by mapping over the reference sequence about 595.000 Tocai mate-pair Sanger reads corresponding to a sequence coverage of 0.9X and obtained from inserts of average size of 3.8 Kb. SNPs were identified between the two genotypes with a frequency of one every 118 bp and a total of more than 2 million SNPs in non repetitive regions. Structural variations were identified as significant size differences between the Tocai inserts and the corresponding regions of the reference sequence as identified by the pair-end reads. We observe several thousands structural variants between Tocai and the reference sequence that could frequently be attributed to the presence of transposable elements in the PN40024 sequence that are missing in Tocai or viceversa and could be attributed to recent insertions into either PN40024 or Tocai. LINE elements within introns appear to be especially polymorphic between the two genotypes. The computational analyses are experimentally validated by gel sizing of Tocai inserts and PCR analysis of transpo sable element insertion sites. The finding of SVs between two grapevine varieties confirms the dynamic nature of the plant genomes, revealing that transposable element activity is an important source for the generation of genetic diversity.

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Poster Abstract – D.12

ANALYSIS OF GENETIC AND EPIGENETIC EVENTS DURING GROWTH AND SENESCENCE IN GRAPE CELL CULTURES

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cell cultures, epigenetic, senescence, Vitis

Senescence is a highly-regulated process, difficult to characterise in planta because of its complexity. By using grapevine cell cultures as experimental system, we are trying to identify genes involved in the regulation of senescence. In view of the difficulties encountered in studying the entire plant, a tree with a long reproductive cycle, the use of cell cultures is instrumental for reaching our goal, but the results acquired in cell cultures will be applied to processes in planta.

Cell cultures represent an ideal system for the determination of the effects of epigenetic modifications on growth and ageing. DNA methylation and post-transcriptional modifications of histones represent the best characterized epigenetic regulation mechanisms in plants. Cytosine methylation occurs in CpG sequence context both in higher plants and animals, but plants differ from animals for significant levels of methylayion at symmetric CpNpG. Two essential roles have been ascribed to DNA methylation: defending genome against transposons and regulating gene expression. The N-terminal and C-terminal tails of histones are subject to post-translational modifications, such as acetylation, methylation, phosphorilation, ubiquitination and others. These covalent modifications are able to modulate chromatin structure and transcription, both directly causing structural changes to chromatin and indirectly recruiting protein complexes that can read these marks and elicit a response. In particular we are studying the involvement of cytosine methylation and histone modifications on growth and aging and their relationships with the transcription and/or silencing of specific set of genes. Initially, genes modulated during cell growth and senescence have been identified in a Köber variety cell line. Among these genes, ten of them were analysed in more details. Subsequently, the pattern of expression of each gene was compared with its methylation status through a DNA McrBC digestion followed by a PCR analysis.

Subtle changes in chromatin structure might be required for fine-tuning of gene expression. For this reason we analysed the heterochromatic histone H3 methylation marks in growing cell cultures by using specific histone methylation antibodies (H3K4,H3K9,H3K27) in an immuohistochemical assay performed on nuclei of cultured cells and in westernblot analyses perfomed on proteins extracted from cells at different physiological stages. Other histone marks will be analyzed in further experiments. Our future aim is to develop epigenetic protocols, such as ChIP and ChIP-seq, for elucidating the roles of heritable traits that do not depend on the primary sequence of DNA but contribute to regulate senescence in grapevine.

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Poster Abstract – D.13

IN SILICO CHARACTERIZATION OF GRAPEVINE STILBENE SYNTHASE MULTIGENIC FAMILY

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stilbene synthase, grapevine, bioinformatic, resveratrol, Vitis vinifera

Grapevine represents one of the major agronomically interesting species on a world scale. The always growing economic relevance of this species and the challenge to reach higher and higher excellence levels regarding productivity and quality has led to an intensification in physiology and genetic studies in the last decades. On August 26th 2007 a great step forward in biology field and grapevine research was done: the first detailed grape genome draft was published on "Nature" journal and a few months later a second draft of a heterozygous grapevine variety was presented on "Plus One" journal.

The Grape-genome public issuing represents a fundamental goal *per se*, but, at the same time it is a formidable starting point for several studies aimed at understanding the gene function and variation in this species. The present work rises from this awareness, and deals with the bioinformatic characterization of the stilbene synthase (STS), an important grapevine multigenic family. Stilbene synthase enzymes catalyze the last metabolic step in the biosynthesis of simple stilbenes (cis- and trans-resveratrol). Resveratrol belongs to the phytoalexins family, showing its role in plant resistance response to biotic and abiotic stresses. Resveratrol is also present in wines and beneficial effects on human health have been consistently reported. Based on interpretation supplied with grapevine genome, the stilbene synthase family includes as many as 43 members. All representative hits that in the grape genome matched with sequences belonging to this family were analyzed. The existence of an organization in smaller sub-families potentially linked to differential responses to various elicitations or to specificity in tissue localization were investigated. Multiple alignments of all these sequences and selection of all those showing a functional catalytic site have let us to design several member-specific primers to amplify 23 different STS members and to study their expression pattern in different organs and tissues and under different elicitations, such as downy mildew infection and UV exposure.

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Poster Abstract – D.14

THE GRAPEVINE TRANSCRIPTOME: BERRY RIPENING AND WITHERING

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grape, gene expression, ripening, withering

Grapevine (*Vitis vinifera* L.) berries undergo considerable physical and biochemical changes during ripening and withering processes.

The main known changes are the chlorophyll degradation, the softening of the berry, the exoses metabolism in the vacuole, the total volume increase, the organic acid catabolism, the appearance of the skin colour (in coloured varieties) and the production of aromas. The withering process is characterized by berry dehydration and an increase in sugar concentration, enriching the wine with higher alcoholic content and particular flavour properties. The winemaking of withered berries is a practise commonly applied to Amarone and Recioto production.

The molecular processes that occur during withering are still poorly understood, therefore detailed transcriptomic analysis of post-harvest grape berries using microarray can be applied to identify the genes involved in a such biological process to select markers that can be used to follow the drying process.

The grapevine transcriptome of berries of *Vitis vinifera* cv. Corvina (red variety, clone 48), sampled during 2006 season covering seven stages from pre-veraison to complete off-plants withering, was analysed using a 25,471-gene chip, named GrapeArray1.2. Expression data were subject to statistical analysis., ripening and withering genes were clustered and grouped into metabolic pathway. This experiment has made a significant contribution to understanding the molecular basis of grape berry withering and may help to identify useful markers for withering processes.

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Poster Abstract – D.15

REGULATION OF ANTHOCYAN SYNTHESIS AND VACUOLAR pH IN DEVELOPING GRAPEVINE BERRY

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grapevine, berry, anthocyanins, vacuolar pH

The grapevine berry has the ability to synthesize many secondary metabolites, some of which are important components towards colour and flavour that define a particular wine.

Phenolics, a class of secondary metabolites, take part in defining colour and some flavour aspects of the berry.

In grapevine berry colour is obtained by anthocyanin synthesis, and colour intensity is controlled by the acidification of the cellular vacuole. In fact the absorption spectrum of anthocyanins depends on pH and therefore skin colour should be influenced by vacuolar pH.

The genetic of flower colour has been characterized in the *Petunia hybrida*. Genetic evidences demonstrated that in *Petunia hybrida* flower colour is regulated by the expression of genes involved in anthocyanin biosynthesis and in regulation of vacuolar acidification. A model of flower colour has been experimentally developed that consists of AN1 (bHLH protein) which regulates both anthocyanin biosynthesis by interaction with AN2 (Myb protein), and vacuolar acidification pathway by interaction with PH4 (Myb protein) [1,2].

The aim of this work is to answer whether the *Petunia hybrida* flower colour genetic model could be extended to berry colour in *Vitis vinifera* cv. Corvina (clone 48) by the characterization of homologue putative transcription factors identified in the grapevine genome[3].

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Poster Abstract – D.16

STRUCTURAL AND NUCLEOTIDE VARIATION AMONG *POPULUS* GENOMES

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structural variation, gene annotation, transposable elements, Populs

Structural variation has been defined as genomic alteration involving DNA segments longer than 1 kb. These generate genomic variants which can contribute to phenotypic differences between individuals, populations and species.

Here, we report a structural variation study within the *Populus* genus carried out through the sequence comparison of allelic chromosomal regions (totaling more than 800 kb) among the three poplar species *P. deltoides*, *nigra* and *trichocarpa*, which are interfertile and, when intercrossed, produce F1 hybrids with improved performances ("hybrid vigour"). Such a study is attractive in this genus, considered a model system for biological studies on trees, as a huge amount of genetic and genomic resources are steadily accumulating within single species of the genus, but there is a lack of information on inter-species comparative analyses.

In the present analysis, allelic pairs of BAC inserts have been detected within the library *Populusxeuramericana* cv. 'Ghoy', an F1 hybrid of *P. deltoides* x *P. nigra*, then sequenced to 10-times coverage, annotated and compared over distances of 90 to 130 kb between the two poplar species and the *P. trichocarpa* reference genomic sequence.

Surprisingly, especially when considering that we are comparing different species, so far the levels of homology and co-linearity resulted to be very high. As in the *P. trichocarpa* reference genome, the other sequenced genomic regions showed a high gene density (putative genes and gene relics considered), where genes are shared and conserved. On the contrary, transposable elements were rarely present, mainly represented by transposable element remnants and classI-retrotransposons, which resulted to be not shared but corresponding to the major insertion tracts. Insertion/deletions represented the main source of genomic variants among the three species, concerning 15% of the whole sequences and spanning few bases to about 10 kb, while no evidences were found for major translocations, duplications or inversions. In this scenario, still microsatellites, especially AT-rich stretches, and SNPs, with a inter-species frequency of 4,5 % on average, represented abundant classes of variants at the nucleotide level.

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Poster Abstract – D.17

EXPRESSION LEVEL AND GENE STRUCTURE IN *ARABODIPSIS THALIANA* AND *ORYZA SATIVA*

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computational genomics, gene expression, microsatellite, gene structure

The relationships between gene structure and expression profiles were investigated in the dicot *Arabidopsis thaliana* and the monocot *Oryza sativa* genomes. Gene structure was described in terms of introns/exons number and average length, and SSR content (e.g. number, type and length).

Expression levels and profiles were retrieved from published microarray datasets in their raw format and re-elaborated by the use of available and *ad hoc* developed bioinformatic tools. Genes were first classified in HK (housekeeping) and NHK (non-housekeeping) depending on their tissue expression profile. Within such categorization, the differences between highly and low expressed genes were further investigated.

The results are presented contrasting the coding (CDS) *versus* non-transcribed regions (5'-UTR and 3'-UTR), and the whole mRNA *versus* the corresponding introns.

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Poster Abstract – D.18

COMPUTATIONAL GO ANNOTATION OF LARGE ORGAN-SPECIFIC EST REPERTORIES REVEALS COMMON FEATURES OF AFLP TECHNOLOGY FOR NON-MODEL PLANT TRANSCRIPTOMICS

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gene ontology, functional annotation, metabolic pathways, regulatory networks, candidate genes

After 10-year-use of AFLP (Amplified Fragment Length Polymorphism) technology for DNA fingerprinting and mRNA profiling, large repertories of genome- and transcriptome-derived sequences are available in public databases for model, crop and tree species. AFLP-based marker systems have been and are being extensively exploited for genome scanning and gene mapping, as well as cDNA-AFLP for transcriptome profiling and differentially expressed gene cloning. The evaluation, annotation and classification of genomic markers and expressed transcripts would be of great utility for both functional genomics and systems biology research in plants. This goal may be achieved by means of the Gene Ontology (GO), consisting in three structured vocabularies describing genes, transcripts and proteins of any organism in terms of their associated cellular component, biological process and molecular function in a species-independent manner. Our work deals with the computational GO annotation of about 8,000 AFLP-derived EST clones retrieved from both publically available databases and unpublished collections.

Descriptive statistics on the type, size and nature of gene sequences obtained by means of AFLP technology were preliminary calculated. The gene products associated with mRNA transcripts were then classified according to basic terminology of the three main GO vocabularies for either single species or botanic families. The adopted Blast2GO software was shown to represent a comprehensive bioinformatics solution for an annotation-based functional analysis. A comparison of the functional content of cDNA-AFLP records sorted by plant organs was also performed by splitting the sequence database into monocots and dicots and by comparing them to all annotated EST datasets of rice and Arabidopsis, respectively. Our findings demonstrated that reliable GO annotations of AFLP-derived sequences can only be gathered through the optimization of the experimental steps and the statistical parameters adopted. Finally, organ-specific cDNA-AFLP sequences from dicots and monocots were used to query the Arabidopsis and rice proteomes, respectively, and the two output sequence collections were then linked to the original microarray probe datasets provided by Affimetrix. The vast majority of our transcript-derived fragments were successfully incorporated in biosynthetic pathways and regulatory networks by using the MapMan software. Qualitative metabolism maps along with regulatory and biosynthetic overviews were developed for specific plant organs across botanic families leading to the definition of key functional categories and groups. Such an exhaustive annotation seems to offer a suitable platform for performing functional genomics and selecting candidate genes, particularly useful in non-model species. According to the whole set of computational-inferred GO annotations, the AFLP technology generates thorough information for angiosperm gene products and shares common

features across angiosperm species and families. Actual possibilities of using AFLP-derived tags on cDNA fragments produced directly by sequencing-by-synthesis technologies opens up the possibility of not only identifying very large numbers of expressed genes, but also retrieving large-scale SNP collections. The utility of this technology for structural and functional genomics in plants can be implemented by serial annotation analyses of genome-anchored fragments and organ/tissue-specific repertories of transcriptome-derived fragments.

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Poster Abstract – D.19

IDENTIFICATION AND VALIDATION OF REFERENCE GENES FOR QUANTITATIVE RT-PCR NORMALIZATION IN WHEAT

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wheat, real time RT-PCR, normalization, housekeeping genes, reference genes

Reference genes with highly uniform expression across different tissues, developmental phases, environments and experimental treatments are required both in advanced (microarray and real time PCR) and traditional (northern) methods of gene expression evaluation and transcript quantification. In plants the search of new reference genes is very limited, whereas most studies concern the evaluation, comparison and validation of well-known reference genes in different species and experimental conditions. Usually the reference genes have been chosen for their known or suspected housekeeping roles in basic cellular processes, such as cell structure maintenance or primary metabolism. However, the variation observed in transcription levels of most housekeeping genes hinders their effective use in gene expression analyses. Cultivated bread wheat (*Triticum aestivum* L.) is an allohexaploid species (AABBDD) with three very large homoeologous genomes, each comprising seven pairs of chromosomes. Since the wheat genome is too large to be entirely sequenced in the near future, structural and functional analysis of the wheat transcriptome is particularly important. The screening of the whole wheat transcriptome has recently been made possible by the availability of an Affymetrix GeneChip Wheat Genome Array containing 61,127 probe sets (55,052 transcripts), likely covering half of the wheat expressed genes. Since qRT-PCR is necessary for array validation and in-depth expression studies, it is important to identify reliable internal control genes suitable for many experimental conditions. Using the terms “wheat”, “gene expression”, and “real time RT-PCR” combined by the Boolean operator “AND” we performed a PubMed search of articles published from January 1996 to March 2008. We had access to 26 articles that used 16 different reference genes. Remarkably, genes encoding 18S rRNA (8 times, 30%), actin (7 times, 27%) and alpha-tubulin (5 times, 19%) have been used in about two third of the studies, whereas other cited reference genes accounted for 4 to 11%. For the normalization of the expression data multiple reference genes have been used in five studies only, whereas in 16 researches single reference genes have been used, presuming their stability of expression without any preliminary validation. The lack of validated reference genes for expression studies in wheat, evidenced by our literature survey, clearly emphasizes the importance of a systematic study for identifying more reliable control genes.

In this study several novel candidate reference genes suitable for gene expression normalization in wheat were identified by a cross search for stable expression in Unigene and TIGR databases. We selected 38 genes representing different functional classes, whose expression was assessed by qRT-PCR in RNAs from 18 wheat tissues and floral organs and in seedlings exposed to low and high temperatures. Additionally, we carried out a comprehensive evaluation of the expression patterns of the actin and alpha-tubulin gene families, which have commonly been used as controls for normalization of gene expression in wheat. Quantitative RT-PCR analyses were

performed by primer pairs designed to target either single genes or two or more members of the same gene family. The expression stability of the genes was estimated by three statistical approaches: 1) cycle threshold (Ct) variation range and coefficient of variation; and 2) geNorm and 3) Norm Finder programs. Finally, we showed that the use of unsuitable reference genes caused a wrong evaluation of relative expression levels of the genes encoding a MADS-box and a PDI-like protein in the analysed tissues.

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Poster Abstract – D.20

STRENOUS EXERCISE IN ATHLETE HORSES: qRT-PCR OPTIMIZATION AND STRESS RELATED GENES EXPRESSION PROFILING

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exercises, horse, qRT-PCR, MMP-1, IL-8

Stress response is a critical factor during training of athlete horses and is critical for obtaining better performance and safeguarding the animal's welfare.

In order to investigate the molecular mechanisms underlying this process, peripheral blood mononuclear cells (PBMCs) were chosen as the candidate cell type to study the physiological changes connected to exhaustive exercise.

Quantitative real-time PCR (qRT-PCR) is the technique of choice to detect modifications in transcription levels of specific genes in a reliable and reproducible manner; however, for an appropriate application are required reference genes whose level of expression is not affected by the test, general physiological conditions or inter-individual variability.

For this reason the expression of nine potential reference genes was evaluated in ten endurance horses during strenuous exercise. These genes were tested by qRT-PCR and ranked according to the stability of their expression using three different algorithms (implemented in *geNorm*, *NormFinder* and *BestKeeper*). Succinate dehydrogenase complex (*SDHA*) and hypoxantine phosphoribosyl-transferase (*HPRT*) always ranked as the two most stably expressed genes.

Subsequently quantitative real-time PCR (qRT-PCR) was performed to detect modifications in transcription levels of Matrix Metalloproteinase-1 (*MMP-1*) and Interleukin 8 (*IL-8*) genes using *SDHA* and *HPRT* as house-keeping genes. The regulation trend of these two genes - revealed among others with a gene discovery approach (cDNA-AFLP) - was confirmed in a larger sample (10 horses) and statistical significance was determined for each assay.

IL-8 expression increased in the race samples ($P<0.001$) and after 24h is was still greater than the basal value ($P<0.05$) even if was lower than the race level ($P<0.001$). *MMP-1* expression increased greatly after the race ($P<0.001$), and after 24h was still higher than the basal one ($P<0.05$), but was lower than the race level ($P<0.001$). *IL-8* showed a similar trend of expression to *MMP-1*, up-regulated immediately after the race, confirming our previous findings with cDNA-AFLP, enhancing the hypothesis that *MMP-1* and *IL-8* are both involved in exercise induced stress.

An attractive hypothesis about their role in the PBMC response to physical stress, besides their relation with inflammatory response, is the involvement in lymphocyte trafficking and in the recruitment of stem progenitor cells from bone marrow, processes that have been recently shown to be related to exercise-induced stress.

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Poster Abstract – D.21

AWN DEVELOPMENT IN BARLEY: DATA INTEGRATION FOR EFFECTIVE VIRTUAL CROP MODELLING

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Awn, barley, Crop Systems Biology

Crop Systems Biology is a promising approach to fulfil challenges in improving complex traits. It combines modern functional genomics and traditional sciences approaches, such as crop physiology and biochemistry, to understand phenotype at the crop level (e.g. grain yield). Data integration thus plays a fundamental role in systems-based approaches and numerous studies are underway to deal with this issue.

Our study shows an example of such a working strategy for the analysis of awn development in barley. The role of awn photosynthetic activity, particularly during stress periods, on grain yield has been largely proven: as a consequence awn characteristics have received quite a lot of attention from the breeders.

Here, a mutant phenotype with a modified awn shape and increased awn area has been utilize as a black box in comparison with its wild type counterpart. System responses to both genotype and environment changes are being evaluated as a basis for application of virtual crop modelling.

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Poster Abstract – D.22

SEQUENCE AND ANNOTATION OF THREE GENOMIC LOCI OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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genomic loci, DNA sequence, Helianthus annuus, repeated sequences

The sunflower (*Helianthus annuus* L.) has a large-sized genome because of a high content of repetitive DNA. To date, the knowledge of the repetitive component of the sunflower genome is based on the sequence and analysis of a small-insert genomic DNA library, that allowed the discovery of many repetitive DNA families (especially transposable elements). However, such an approach did not allow isolating full-length elements and studying dynamic and structural aspects of the sunflower genome evolution. For example, since the scarcity of long range genomic sequences, it is not known how repetitive elements are arranged in the chromosomes and the extent of dispersion of genes among non-coding sequences.

We report the annotation of three large regions from the genome of the sunflower, screened from a BAC library by hybridization using three putative single-copy genes, encoding a lipid-transfer-protein (LTP), a Z-carotene desaturase (Z-DES), and a dehydrin (DHN). The three BAC clones account for 136, 97, and 131 contiguous Kb, respectively.

Beside LTP, Z-DES, and DHN, other protein encoding genes were identified, accounting on their whole for 27 over 363 Kb. The BAC clone carrying the LTP gene revealed that this gene is present in five copies of different length and sequence.

Several families of repetitive DNA were identified. *Gypsy* retroelements were by far the most represented, followed by *Copia* retrotransposons. Four non-autonomous LTR-retrotransposons (the so-called LARDs) were found for the first time in the sunflower. Other repetitive families included non-LTR retrotransposons and DNA transposons. In many cases, complete elements were discovered.

Our analyses also provided the first insight into the local organization of the sunflower genome showing nests of retrotransposons inserted into each other and allowing the estimation of retroelement insertion ages. Different waves of retroelement mobilization during the evolution of this species and the occurrence of very recent retroposition events are suggested.

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Poster Abstract – D.23

CHARACTERIZATION OF HACRE1, A COMPLETE *COPIA* RETROTRANSPOSON OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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copia retrotransposon, long terminal repeats, Helianthus annuus

Retrotransposons or their remnants constitute large portions and have largely contributed to the structure of eukaryotic genomes. In sunflower (*Helianthus annuus* L.), recent studies showed that Gypsy and Copia retrotransposons account for large portion of the genome and that retrotransposon amplification have largely contributed to the speciation of *Helianthus* genus, being strongly activated after interspecific hybridization. However, no complete retrotransposon sequences are available.

After sequencing and annotation of a sunflower BAC clone, we have obtained and analysed, for the first time, an entire sequence of a Copia retrotransposon of sunflower, named HaCRE1 (Helianthus annuus Copia RetroElement 1), which is 8,511 bp long. HaCRE1 belongs to the Superfamily Copia retrotransposons by its protein domain order and sequence similarity to other Copia elements of dicotyledons and by phylogenetic analyses.

HaCRE1 carries 5'- and 3'-long terminal repeats (LTRs) 919-bp and 931-bp in length, respectively, flanking an internal region of 4661 bp. HaCRE1 has LTRs identical in their sequence, excluding two deletions of 7 and 5 nucleotides in the 5'-LTR. Availability of both complete LTRs allows a precise estimate of insertion time of HaCRE1. Insertion time estimates are based on the occurrence of nucleotide substitutions between LTRs, that are supposed to be identical at the RE insertion, using a nucleotide substitution rate of 1.3×10^{-8} substitutions per site per year. If only one substitution had occurred in the 919 sites of LTR, the insertion of the element should date 83703 years ago. This suggests that HaCRE1 inserted in the last 83703 years. The isolated sequence contains a complete ORF, with only one complete frameshift, i.e., no additional stop codons were found beside the regular stop at the end of the pol gene. The absence of non sense mutations agrees with the nearly complete convergence between LTRs, confirming that HaCRE1 is recent.

Slot blot hybridization experiments showed that the haploid genome of sunflower (HCM inbred line) contains about 160 copies of HaCRE1. The transcription activity of HaCRE1 was analyzed by semi-quantitative reverse-transcription PCR (RT-PCR) and sequencing in different plant organs and under different culture conditions. HaCRE1 resulted constitutively expressed, probably related to the occurrence, in the LTRs, of many putative regulatory cis-elements.

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Poster Abstract – D.24

A PLANT GENOME ANNOTATION PIPELINE FOR DISEASE RESISTANCE GENES

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resistance gene, plant disease, database, bioinformatic

Plant disease resistance genes (R-Genes) are an important class of genes which are well characterized at the molecular level. These genes play a key role in the recognition of the products of avirulence (Avr) genes from pathogens and in the activation of plant defence responses. So far, in the Solanaceae family, 29 R-genes have been isolated and characterized. Using these genes as reference sequences we have built a bioinformatic pipeline for fishing R-gene in large dataset (whole genomes, "Unigene" clusters and NCBI sequence collection). Combing phylogenetic data with conserved domain dissection 400 sequences were extrapolated from NCBI dataset. Sequences have been download ordered and stored in our SRG database (http://srg.cbm.fvg.it/index.php). A graphic view of putative cDNA and protein sequence structure with indication of resistence gene domain location is provided to facilate data anallysis. With this procedure we were able to fish and order all Solanaceae putative resistance sequences accurately and quickly. Our future goal is to build an automatic system for fishing sequences related to disease in all the new sequenced plant genomes.

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Poster Abstract – D.25

MICROARRAY ANALYSIS OF GENE EXPRESSION IN TOMATO PLANTS INFECTED BY DIFFERENT COMBINATIONS OF CUCUMBER MOSAIC VIRUS AND ITS SATELLITE RNAs

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tomato, Cucumber mosaic virus, defense response, microarray, Combimatrix

An analysis of transcriptional changes in tomato plants, induced by the infection of Cucumber mosaic virus (CMV), alone or in combination with satellite RNA (satRNA) variants, has been undertaken by microarray analysis. The analysis was performed a new CombiMatrix platform, on a tomato chip carrying 20200 specific probes from assembly of Tentative Consensus of the last Tomato Gene Index, release 11.0 (June 21, 2006).

Solanum lycopersicum cv. UC82 plants were infected with CMV-Fny or with CMV-Fny co-inoculated with three different satRNAs (benign: CMV-Fny/Tfn-satRNA; stunting: CMV-Fny/TTS-satRNA; necrogenic: CMV-Fny/77-satRNA). Mock-inoculated plants were used as controls. Gene expression was examined at 2 and 9 days post-inoculation. 1179 genes were modulated in at least one condition. CMV-Fny, without any satRNA, provoked wide transcriptional changes, affecting about 80% of modulated genes. Core sets of transcripts coherently modulated (either up-or down-regulated) by all infections or by the 3 CMV/satRNA combinations were identified.

To identify genes that might account for the different symptoms observed in the different CMV/satRNA co-infections, the transcriptional effect of each single CMV/satRNA combination was compared with the effect of CMV-Fny: all satellites determined a clear down-regulation of genes that are expressed in CMV-Fny-infected plants, especially at 2 days p.i., while interesting differences could be identified between gene expressions specifically associated to each CMV/satRNA infection.

Grouping of differentially expressed genes into putative functional categories revealed some peculiarities of all examined interactions, such as a very small percentage of photosynthesis-related genes and, conversely, a consistent modulation of resistance-related and signal transduction–related genes. Modulated genes related to “Lipid metabolism” and to “Nucleic acid metabolism” were also well represented. This work might serve as a basis to identify candidate genes with a functional role in susceptibility and symptom determination.

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Poster Abstract – D.26

TRANSCRIPTIONAL CHANGES IN GRAPEVINE IN RESPONSE TO BOIS NOIR INFECTION

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Vitis vinifera, Bois Noir, expression profiling, Affymetrix oligonucleotide array

Bois Noir, a yellows disease of grapevine (*Vitis Vinifera*), is caused by a non cultivable phloem-limited phytoplasma. The physiology of its pathogenesis remains still poorly understood, and very few informations exist about the interaction with the plant. To better clarify this aspect, Affymetrix GeneChip® oligonucleotide arrays have been used to identify differentially expressed genes between infected and recovered samples from 'Chardonnay' cultivar, as well as between infected and healthy samples from 'Incrocio Manzoni' cultivar.

Nested-PCR reaction was performed to evaluate the disease status of each sample collected in the experimental field, subject to natural inoculation. Incrocio Manzoni in the field conditions showed a moderate level of tolerance, both in terms of disease severity and of number of infected plants, while Chardonnay cultivar was strongly susceptible to the disease.

Results showed that expression levels of few hundred genes were altered in infected plants, with effects on various metabolic pathways.

Genes involved in photosynthesis and lipid metabolic process were exclusively suppressed in Chardonnay plants. In both Chardonnay and Incrocio Manzoni infected plants, genes involved in cellular component organization and biogenesis, protein metabolic process and nucleobase, nucleoside, nucleotide and nucleic acid metabolic process were inhibited, while carbohydrate metabolic process were strongly affected, with some genes induced and some repressed.

The present work gives a first and detailed insight into the functional genomics of the response of grapevine to the emerging yellows disease Bois Noir.

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Poster Abstract – D.27

ANALYSIS OF GRAPEVINE GENE EXPRESSION IN RESPONSE TO *PLASMOPARA VITICOLA* BY COMBIMATRIX MICROARRAY

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grapevine, Plasmopara viticola, defense response, microarray, Combimatrix

A comprehensive analysis of transcriptional changes associated to the infection process of *Plasmopara viticola* in susceptible (*Vitis vinifera* cv. Pinot Noir) and resistant (*Vitis riparia* cv. Gloire de Montpellier) grapevine genotypes has been undertaken by microarray analysis, at different time points after infection. The analysis was performed on Combimatrix platform at Verona University, on a Grape chip carrying 24562 specific probes in triplicates from assembly of Tentative Consensus of the last TIGR *Vitis vinifera* Gene Index release 5.0 and from non reduntant genomic sequences produced by the genome annotation in the International Grape Genome Project.

Leaves of resistant and susceptible grape plants grown *in vitro* were infected with *P. viticola* or treated with distilled water as a control, and collected at 12 and 24 hours post-inoculation (hpi). Hybridisations were carried out with samples deriving from three independent biological replicates. Differentially expressed genes were selected using the multi experiment Significance Analysis of Microarray test, and gene clustering was performed using Genesis software.

Results showed an increase in the steady state level of more than 1500 genes in the resistant genotype and of about 300 in the susceptible Pinot Noir, as ealy as 12 hpi. While in the resistant genotype the transcriptional up-regulation remains sustained also at 24 hpi, the response of the susceptible genotype is characterized by a massive down-regulation of gene expression, which could support the hypothesis of a pathogen-driven suppression of general defence responses. Genes have been assigned to putative functional categories according to the Gene Ontology tool. Candidate genes possibly involved in signal transduction during early phases of infection will be the object of future investigations.

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Poster Abstract – D.28

MAPPING QTLs FOR LEAF RUST RESISTANCE IN THE MODEL PLANT *BRACHYPODIUM DISTACHYON*

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Brachypodium distachyon, Puccinia brachypodii, leaf rust resistance, model plant

Brachypodium distachyon has been proposed as a model system for functional genomics in Triticeae, and thus in wheats and barley, because of its biological features and compact genome size. This new model plant has been employed to build a linkage map as a genetic resource for the scientific community, and to study the genetics of resistance to leaf rust fungi caused by *Puccinia* spp., that are important diseases in cereals in many regions of the world, causing severe yield losses.

Five *Brachypodium distachyon* inbred lines were challenged with different *Puccinia* spp. in order to identify leaf rusts able to elicit disease symptoms. An interesting variation in responses was observed following challenge of *B. distachyon* lines with a *Puccinia brachypodii* isolate. In all *Brachypodium* lines the fungus developed sporulating lesions. Different levels of partial resistance, defined as a resistance that results in reduced epidemic development despite a compatible infection type, were observed between the five lines. In some lines, however, the pustules were associated with rather extensive necrosis of plant tissue.

A genetic linkage map of *B. distachyon* covering 1139 cM, with 171 AFLP loci, was constructed by using an F2 population of *B. distachyon* derived from a cross between the leaf rust susceptible line Bd1-1 and the partially resistant line Bd3-1. The F2 plants were quantitatively phenotyped for resistance to *P. brachypodii* at seedling and adult plant stages in the greenhouse. QTL analysis via MQM mapping method detected 3 QTLs for resistance at seedling stage and 1 QTL at adult plant stage. The QTL picture of partial resistance to leaf rust in *B. distachyon* could allow to compare the genetic architecture of quantitative leaf rust resistance in *Brachypodium* and that in major cereals like barley and wheat.

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Poster Abstract – D.29

ISOLATION AND MOLECULAR MAPPING IN *CAPSICUM ANNUUM* OF THE TOMATO *VE*2 GENE CONFERRING RESISTANCE TO *VERTICILLIUM* SPP.

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Capsicum spp., Verticillium spp., Ve gene, molecular mapping

Verticillium wilt is a widespread fungal disease caused by two soil-borne pathogens: V*erticillium dahliae* and *Verticillium albo-athrum*. They are responsible of important yield and quality losses in a wide range of crops. Few strategies are at present available for the control of these pathogens.

To date tolerance to Verticillium wilt has been found in *Capsicum chinense, C. frutescens* and *C. baccatum* genotypes, while no tolerant lines have been identified in *C. annuum*, the most commercially important species.

In tomato the genetic resistance is conferred by two inverted independent genes: *Ve1* and *Ve2*, which have been mapped on the short arm of chromosome T9.

Our aim was to isolate sequences homologues to *Ve1* and *Ve2* in pepper and identify their location in three previously developed maps, based on the following intra-specific *C. annuum* progenies: (i) 297 F6 RILs from the cross 'Yolo Wonder' x 'Criollo de Morelos 334' (YC); (ii) 114 doubled haploids from the cross 'Perennial' x 'Yolo Wonder' (PY); (iii) 101 doubled haploids from the cross 'H3' x 'Vania' (HV).

C. annuum leaves were used for mRNA extraction and cDNAs generated by reverse transcription. Primer were designed on the tomato *Ve1* (AF272367) and *Ve2* (AF365929) gene sequences and applied for amplifications of pepper homologous cDNAs. PCR products (860 bp) were obtained only with *Ve2* primers. After sequencing, a nucleotide database search (blastn) revealed high similarity (87% identity) with the tomato *Ve2* gene.

Specific pepper primers were designed for analysing the allelic forms of the pepper homologous *Ve2* gene (*CaVe2*) in the five parents of the mapping populations. A SNP between 'Yolo Wonder' and 'Criollo de Morelos 334' was identified, and a specific co-dominat marker developed by means of the software dCAPS Finder 2.0 (http://helix.wustl.edu/dcaps/dcaps.html). Segregation analyses made possible to localize *CaVe2* locus on chromosome P9, in a position analogous to the one of the tomato *Ve* loci.

After successful full length *CaVe2* isolation, new primer pairs were designed for the development of specific co-dominant markers useful for mapping the gene in PY and HV progenies; segregation analyses are at present underway.

Further studies are in progress to evaluate the role played by this gene in the pepper tolerance to *Verticillium*.

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Poster Abstract – D.30

GRAPEVINE STILBENE SYNTHASE EXPRESSION AND RESVERATROL SYNTHESIS FOLLOWING DOWNY MILDEW INFECTION

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Vitis vinifera, Plasmopara viticola, stilbene synthase, grapevine germplasm, resveratrol

In the *Vitaceae* family, phytoalexins constitute a rather restricted group of molecules belonging to the stilbene family. Stilbenes represent a group of natural phenolic compounds including *cis*- and *trans*-resveratrol (3,4',5-trihydroxystilbene), resveratrol glycosides, resveratrol oligomers (viniferins) and pterostilbene.

Resveratrol plays a relevant role in the resistance of grapevine challenged by fungal pests, and it is also the major compound accumulating in response to this stress.

Within the *Vitis* genus genes encoding stilbenes are widespread, even in susceptible species and varieties, and the understanding of genes involved in stilbene biosynthesis, as well as the elucidation of their regulation, is rapidly expanding. Stilbene production is elicited by fungal cell walls, polysaccharide fragments and other fungal molecules which induce *de novo* synthesis of enzymes of the general phenylpropanoid pathway. In particular, the last step is catalyzed by stilbene synthase (STS), which produces simple stilbenes (*cis*- and *trans*-resveratrol) from one *p*-coumaroyl-CoA and three malonyl-CoA molecules.

The plant material subject of the present study comprises two international cultivars (Cabernet sauvignon, Chardonnay), three genotypes indigenous of the Euganean area (Friularo, Pattaresca, Marzemina bianca) and 12 interspecific hybrids deriving from a cross between Chardonnay and Bianca, susceptible and resistant to *Plasmopara viticola* respectively.

Healthy young leaves were collected for each plant, and leaf discs were produced and artificially inoculated with a suspension of *P. viticola* sporangia. Total RNA isolation was performed at 0-, 2- and 6dpi, and stilbene synthase transcript level measured by Real Time RT-PCR.

At the same time points, resveratrol content in the infected discs was quantified by HPLC, and macroscopic syntoms were scored, in order to finally relate the stilbene synthase expression to the resveratrol synthesis and the infection's macroscopic display in the course of *P. viticola* infection.

The outcomes of the analyses highlighted differences among the grapevine genotypes concerning both stilbene synthase expression and resveratrol synthesis in leaves challenged by downy mildew.

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Poster Abstract – D.31

FUNCTIONAL CHARACTERIZATION OF AN E3 UBIQUITIN LIGASE INVOLVED IN PLANT RESPONSE TO ABIOTIC STRESS

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ubiquitylation, E3 ligase, abiotic stress, Two-hybrid system

Protein ubiquitylation is a post-translational modification that targets protein substrates for 26S proteasome-mediated degradation. It is based on the covalent attachment of the 76-amino acid eukaryotic molecule, ubiquitin, to substrate proteins. Protein ubiquitylation plays a key role in a wide variety of cellular processes such as hormone signalling, DNA repair, biotic and abiotic stress response, cell cycle regulation. Ubiquitin conjugation is a multistep reaction, sequentially involving three enzymes referred to as E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme) and E3 (ubiquitin ligase). In *Arabidopsis thaliana* more than one thousand of genes code for E3 ubiquitin enzymes that specifically recognise target proteins. In a previous work we isolated an *E3* ubiquitin ligase early induced during cold/light stress in durum wheat; an ubiquitylation assay was carried out to test its functionality *in vitro*. To identify potential ubiquitylation targets during abiotic stress response, several approaches have been initiated in *Triticum durum* and *Arabidopsis* based on the identified *E3* gene. A wheat cDNA library from cold treated leaves of *Triticum durum* has been produced and screened by two-hybrid system to isolate potential E3 interactors and ubiquitylation targets. Transgenic *Arabidopsis* plants overexpressing the *Arabidopsis* homologous E3 enzyme fused to the TAP tag has been developed to isolate protein complexes containing our E3 ligase and to determine subcellular localization of E3 enzyme. An *Arabidopsis* K.O. line for the same gene has been obtained for future evaluation under various abiotic stress condition. Preliminary results of this work will be shown.

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Poster Abstract – D.32

STUDY OF GENOMICS AND PROTEOMIC RESPONSE TO ABIOTIC STRESS IN TOBACCO CELL LINE

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Methylation DNA, Nicotiana tabacum, stress tolerance

Plant growth and productivity depend on the interaction of genotype with various external factors. Every time that there is a drastic change of environment factors a stress occurs. The principal goal of this research is to study the characterization of cellular response, analysis of protein expression and DNA epigenetic modifications changes during abiotic stresses.

Nicotiana tabacum L. cell cultures have been used and Heat shock has been used as model system of abiotic stress.

The work has been performed on cellular lines of tobacco (TBY-2) subjected to different timing of abiotic stress (Heat-Shock at 35°C).

The Southern Blot analysis have been used to verify changes of methylation pattern in cellular lines when subjected to different timing of stress temperature. We digested DNAs by using some isoschizomeric enzymes, differently sensitive to the pattern of methylation (HpaII/MspI; MboI/Sau3AI). Afterward, they have been hybridized using, as radioactive probes, regions of repeated DNA (rDNA 5S, rDNA 18S, Tto1 retrotrasposon, repeat BamHI DNA etc)(Wade, 2004).

Moreover, in order to assess the establish of oxidative stress and damage (determination of production of reactive oxygen and nitrogen species and protein oxidation and lipid peroxidation), study level and redox state of ascorbate, glutathione, pyridine nucleotides and level of SH protein group oxidation have been carry out.

These analyses quickly allowed us acquiring useful details about the variation of the methylative state of the DNA in the cells during the time course.

This kind of experimental approach allows us to quickly and clearly highlight both the effects of applied stress on the genome methylation state and the proteomics response induced.

The analyses conducted so far revealed both there is a clear genome hypomethylation and an increase in total protein, besides we found a drastically change of proteomic dowry, probably due to the increased expression of heat-shock proteins.

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Poster Abstract – D.33

EFFECT OF UV-B DEPLETION ON FLAVONOID CONTENT AND FLAVONOID BIOSYNTHETIC GENE EXPRESSION IN FLESH AND PEEL OF WILD TYPE AND *hp-1* TOMATO FRUITS DURING RIPENING

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UV-B, flavonoids, gene expression, tomato

Flavonoids are a class of plant polyphenols that comprises up to 5000 different molecules, many of which have shown to be useful for human health. In plants, generally, they are ascribed to have an important role in plant defense, especially to ultraviolet radiation.

Tomato is an important crop which is worldwide cultivated and eaten. It is the source of some health-beneficial secondary metabolites. The most represented ones are carotenoids but tomato fruit has proven a modest source of flavonoids too, especially in peel.

Little is known about the possibility of enhancing flavonoids in tomato fruits by modulating light quality instead of biotechnological approaches and, therefore, aim of this study was to evaluate the effect of ultraviolet B radiation depletion on the expression of some genes involved in flavonoid biosynthesis and on the accumulation of some flavonoids in two tomato genotypes.

Wild type and mutant *hp-1* tomato plants were grown until complete fruit ripening under two different light conditions: whole sunlight spectrum and sunlight spectrum deprived of the UV-B region (280-320 nm). Flesh and peel of fruit were harvested at three different ripening stages and samples analyzed to evaluate the influence of the genotype and UV-B depletion on the flavonoid content and the expression of some flavonoid structural and regulatory genes.

Our analyses indicate that the *hp-1* mutation enhances flavonoid gene expression and accumulation both in flesh and peel. However light quality differentially affects the content of the phenolics analysed and the expression of some of the biosynthetic genes in the two tissues during the ripening process.

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Poster Abstract – D.34

NMR TECHNIQUES COUPLED WITH MULTIVARIATE STATISTICAL ANALYSIS: TOOLS TO ANALYZE *ORYZA SATIVA* METABOLIC CONTENT UNDER STRESSES

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Oryza sativa, NMR, drought, high salinity, PCA

Rice (*Oryza sativa* L.), one of the most important crops in the world, is the staple food for over half the world's population. Among European countries, Italy is the largest rice producer and exporter, with cultivated areas extending over 228,000 ha and production of 1,430,000 tons during 2006.

Even if rice growth on the most productive irrigated lands in the world has reached almost the maximum potential production, the achievement of the optimum yield is made difficult by environmental stresses, such as water deficiency, soil salinity and pathogens attack. The development of new rice varieties with a higher tolerance/resistance to both abiotic and biotic stresses is of great interest also in our country, for the adaptation of rice to suboptimal climate and soil conditions.

Metabolomics is considered an emerging tool for metabolic pattern studies and, together with transcriptomics and proteomics, for gene function identification, under normal and stress conditions. One strategy to identify more suitable varieties might be to compare the metabolic profile of different cultivars and verify a possible correlation between the accumulation of specific metabolites and the level of stress tolerance/sensitivity.

In this work, we have first investigated the metabolic profile in shoots and roots of two rice cultivars (Arborio and Nipponbare) through solid state ^{1}H HR MAS and liquid state NMR experiments. Drought and salt stress experiments on shoots and roots growth showed a higher sensitivity of Arborio seedlings than those of Nipponbare to these abiotic stresses. Moreover, the metabolic content of the same samples was analysed by liquid state NMR coupled with multivariate statistical analysis. Principal Component Analysis highlighted a significant accumulation of amino acids and sugars in shoots and roots under stress conditions and the existence of clear differences between the two analyzed rice cultivars. In particular, Arborio seedlings accumulated a higher concentration of amino acids and sugars than Nipponbare ones. Furthermore, we also obtained preliminary data about rice metabolic changes following infection with the fungus *Magnaporthe grisea*.

This work indicates that NMR technique coupled with multivariate statistical analysis is a powerful tool to assess a possible correlation between differences in metabolic profile and in tolerance/sensitivity phenotype in rice cultivars.

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Poster Abstract – D.35

TOWARD THE FUNCTIONAL CHARACTERIZATION OF THE SUMO PATHWAY DURING THE PLANT RESPONSE TO ABIOTIC STRESSES

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Sumoylation, cold stress, heat stress, proteomics

The transient conjugation of the Small Ubiquitin-like MOdifier (SUMO) protein to target proteins is a post-translational modification playing an influential role in a wide variety of cellular processes, by regulating protein-protein interactions and subcellular location or by antagonizing ubiquitination. In yeast and human, SUMOylation target proteins are factors involved in DNA repair and chromosomal segregation, transcriptional regulators and RNA-binding proteins, cytoskeleton components and nuclear transport factors as well as metabolic enzymes. Mechanistically, SUMOylation involves the sequential action of a SUMO activating enzyme (E1 or SAE), a SUMO conjugating enzyme (E2 or SCE) and eventually an E3 ligase. SUMOylation is conserved in plants and the conjugation system of *Arabidopsis* has been characterised, however a few number of plant SUMO-conjugates have been identified so far and evidence on SUMOylation functions in plant life is limited. Activation of the SUMOylation is a known response of Arabidopsis to abiotic stresses like heat, H2O2 and cold. We assessed the effect of exposure of *Hordeum vulgare* and *Triticum durum* to temperature stresses by the expression profiling of genes of the SUMOylation pathway and by the accumulation of SUMO conjugates. Given that they are potential regulators of the plant molecular response to stresses, in order to obtain their isolation three proteomic approaches have been initiated in *Triticum durum* and *Arabidopsis.* Transgenic *Arabidopsis* plants overexpressing the SUMO conjugating enzyme AtSCE1a fused to the TAP tag have been produced to isolate protein complexes of the SUMOylation pathway. A wheat cDNA library from cold treated leaves has been screened by yeast two-hybrid analysis to isolate potential E2 interactors (SUMO-conjugates and E1/E3 enzymes). An immunoaffinity chromatography by the anti-SUMO1 immunoglobulin followed by LC-MS/MS analysis has been used to identify wheat proteins conjugated to SUMO after heat stress. Preliminary results of these strategies are shown.

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Poster Abstract – D.36

SUGAR BEET RESPONSE TO COLD: A qPCR BASED GENE EXPRESSION PROFILING

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reference genes, cold stress, sucrose metabolism, real-time PCR, Beta vulgaris

The exposure to low temperatures of young sugar beet plantlets may lead to serious losses of sucrose productivity and quality. Technological advances in high-throughput molecular analysis lead to a better knowledge of the complex regulation of gene expression underlying the cold response in *Arabidopsis thaliana*, a model for many other important crop species like sugar beet (*Beta vulgaris*). For sugar beet, specific extensive genomic data are relatively limited. Integrated "dry/wet" approaches, and advanced high-throughput techniques, based on real-time PCR, are able to reveal gene expression fluctuations induced by abiotic stresses treatments, allowing overcome such limits.

Our goal was the gene expression profiling of several sugar beet genes involved in sucrose quality and yield by real-time PCR, in order to investigate how and if cold stress modulates their transcription levels in different organs of the plant. A previous selection of the most suitable reference gene for the species, organ, developmental stage and treatments was necessary to validate the data obtained. After this preliminary selection, seven putative housekeeping genes were characterised, their Ct analyzed by "comparative Ct method" and the index of stability (M) calculated by GeNorm software. Tubulin, rRNA and elongation factor1α were found to be the most suitable reference genes in our conditions and materials, and were therefore employed to normalize the data relative to the target genes analyzed. The expression of twelve target genes was quantified in leaf and roots of young sugar beet plantlets exposed to low temperature treatments; the results indicate organ-specific variability and stress-modulated transcription in some conditions, especially for the genes directly involved in the sucrose biosynthetic pathway (SBSS1, SBSS2, SPS1 and SPS2).

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Poster Abstract – D.37

PHYSICAL MAPPING OF THE BARLEY *Fr-H2* (*FROST RESISTANCE-H2*) LOCUS

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frost resistance, CBF, physical mapping, HICF, contig

A positional cloning effort of one of the major quantitative trait loci that affects freezing tolerance and winter hardiness of barley - Hv*Fr-H2* - where the QTL is linked to the physical sequence of the genome via the fingerprinting of large insert clones, has been undertaken. The *CBF* genes are the best candidates in barley to explain the effects of frost tolerance given by the QTL *Fr-H2*. Determining whether the effect of Hv*Fr-H2*, is the result of a single *CBF* gene, the combined effect of a subset (or all) of the CBFs, or independent by the *CBF* genes remains to be determined.

To address this issue a genomic BAC library of barley (cv. Morex) comprising 313,344 BAC clones was screened with a total of six HvCBF markers, out of the 14 *CBF* genes mapping in this locus. A four-step PCR-based screening protocol was used employing DNA of BAC pools. Using that strategy the first BAC clone addresses were obtained for all CBF markers assayed. To create anchor points between the genetic map and a 'future' physical map of barley, in this region, the fingerprinting (HICF- high information content fingerprinting) of the selected BACs has been performed. After HICF analysis, the selected BAC clones have been assembled into contigs based on the overlapping bands shared by the individual BACs, using FPC V8.5 software. To close the gaps between the assembled clones, additional BACs belonging to the contigs detected, have been screened with the previous six CBF markers, additional CBFs and other markers deriving from rice-barley sinteny investigation. Moreover BAC-end sequencing has also been undertaken for direct linking of assembled contigs. The establishment of a contig between flanking markers is the first aim to clarify the genomic structure of *Fr-H2* QTL region. This will also provide a fundamental resource for detailed comparative analysis of the genomic organization of the locus in other barley cultivars, like cultivar 'Nure' and 'Dicktoo'.

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Poster Abstract – D.38

CHARACTERIZATION OF A NOVEL POTATO GENE CODING FOR A PUTATIVE RNA BINDING PROTEIN INVOLVED IN PLANT RESPONSE TO WATER STRESS

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water stress/adaptation, qRT-PCR, RNA binding protein, knockout mutant, protein localization

Plants respond to abiotic stresses by complex mechanisms involving a wide gene network. In order to preserve productivity of species in adverse environmental conditions is crucial to identify and characterize molecular functions that regulate the stress response and, above all, the adaptative response.

Previously, we reported a transcriptome analysis of potato cells exposed to short (shock) and long- term (acclimation) water stress induced by PolyEthyleneGlycole. Comparison of two different responses was performed by the TIGR 10k potato array challenged with RNA isolated from untreated, PEG-shocked and PEG-acclimated cells (Ambrosone et al. 2006, Transcriptomic and gene expression analysis during water stress in potato. Proceedings of SIGA Annual Congress).

Among the genes consistently induced during long- term water deficit the EST AW906734 coding for gene sato2, acronimous of Salt Tolerance, was identified. Sato2 gene encodes for a protein conferring salt resistance by complementation of a yeast defective mutant (Ros et al. unpublished) and contains a conserved RNA-binding protein domain. We isolated the sato2 coding sequence of *S. tuberosum* by RT-PCR using primers designed on sato2 mRNA sequence of *S. lycopersicum* (BT014404). Sequence translation and BlastP search reveals that the protein is highly conserved with more than 60% identity in several species as *Beta vulgaris, Vicia faba, Spinacia olearia and Arabidopsis thaliana.*

Gene expression of sato2 was investigated in potato cells, leaves and roots by qRT- PCR confirming the gene is responsive to water deficit conditions. A characterization of the A. thaliana sato hortologous gene (Atsato, At4g16830) was carried out. Atsato codes for a RNA-binding protein of 355 aa containing a RGG box with unknown function (www.arabidopsis.org). The gene resulted up- regulated in arabidopsis cells exposed to 50 uM ABA, 150 mM NaCl and 10% PEG. Phenotypic and physiological analysis of Atsato knockout (Atsato KO) indicated that the mutant was severely affected by ABA, NaCl and PEG treatments. In particular, root elongation of Atsato KO was inhibited compared to wt Columbia genotype (Col-0) in medium containing 80 mM NaCl and in GM plates equilibrated with 35% (w/v) PEG solution .To investigate the subcellular localization of the SATO2, transgenic plants overexpressing YFP-SATO fusion protein were obtained. The SATO2::YFP-fluorescence signal revealed SATO2 localizes in the cytoplasm of arabidopsis cells. We utilized 1500 bp sato promoter- GUS fusion to understand the expression patterns of the gene in arabidopsis vegetative and reproductive tissues. GUS expression driven by sato regulatory sequences was found in leaves, stomata, petals, sepals and pollen. Gain of function

and molecular interaction studies are in progress to establish the functional role of sato2 in water stress response.

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Poster Abstract – D.39

COMPARATIVE PROTEOMIC ANALYSIS OF HEAT STRESS ON THE METABOLIC SEED PROTEIN FRACTION IN THE WIDELY GROWN ITALIAN DURUM WHEAT CULTIVAR SVEVO

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durum wheat, heat stress, albumins and gGlobulins, Mass spectrometry

Durum wheat is mostly grown and represents one of the most important crops in Central and Southern Italy and the grain filling occurs between April and May, when sudden increases in temperature may take place. High temperature during grain filling has already been recognized to cause a deviation of expected properties and quality characteristics of bread wheat doughs. This iss a consequence of differential accumulation of gluten proteins that resulted in an alteration of their ratios that, in turn, modify technological properties of doughs.

Wheat grain proteins are typically classified according to their solubility proprieties into albumins (water soluble), globulins (salt soluble) and prolamins (gliadins and glutenins). These latter make up the gluten, and are mostly responsible for rheological properties of wheat doughs. Non-prolamin fractions include proteins with metabolic activity or structural function. Many of these proteins may generate allergies or intolerance in sensitive individuals.

In order to verify the consequences of heat stress on endosperm protein accumulation in durum wheat, we submitted the widely grown cultivar Svevo (5 D.P.A) to two thermal regimes (heat: 37/17°C day/night for five days and control 20/17°C), by producing four biological replicas for each treatment.

Two-dimensional electrophoresis (IEF/SDS-PAGE) was carried out on the metabolic (non-prolamin) fraction. IPG strips (18 cm long) in the pH range 3-10 were used to perform three different technical replicas for each biological replica. Spots were revealed with Coomassie Brillant Blue (CBB) and analyzed with Progenesis SameSpots (Nonlinear Dynamics, UK), in order to identify differentially expressed polypeptides between heat stressed and control plants.

The gel analysis revealed 132 differentially expressed polypeptides. These polypeptides were collected and their identification performed by MALDI TOF and MALDI-TOF-TOF.

Forty seven spots differentially regulated (15% down-regulated and 85% up-regulated) were identified by NCBInr and TIGR Wheat protein database search. The identified proteins are functionally diverse and include: Heat Shoch Proteins (HSP70), proteins related to ATP synthesis, proteins involved in glycolysis, carbohydrate metabolism, and stress defensive related proteins.

Differences found between bread and durum wheat are discussed.

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Poster Abstract – D.40

SHOOTMERISTEMLESS: A GENE INVOLVED IN THE MAIZE SHOOT APEX INITIATION

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shoot apical meristem, Zea mays, plant development

The regulatory mechanism of shoot apical meristem (SAM) initiation is an important subject of investigation in developmental plant biology. The establishment of the shoot apical meristem (SAM) during embryogenesis is a key event in plant development, because the above-ground body plan depends on the activity of the SAM through the production of leaves, axial buds and stem. A genetic approach utilizing developmental mutants is an efficient way investigate on this topic.

Here we describe a mutation of the *shootmeristemless* (*sml)* gene disrubting shoot apical meristem maintenance and lateral organ formation. Introgression of this mutation in different genetic backgrounds has highlighted the epistatic interaction between *sml* and the unlinked *distorted growth* (*dgr)* gene. Seeds homozygous for both *sml* and *dgr* have a shootless phenotype whereas *Dgr/-sml/sml* seeds produce plants with many developmental abnormalities (dgr mutant).

sml lies on the long arm of chromosome 10, and its map position has been defined by linkage analysis with visible and molecular markers. To clone it we have adopted a candidate gene approach. The *SHOOT ORGANIZATION1* (*SHO1*) gene, recently isolated in rice, lies on a sintenic region. A mutant in this gene shows abnormal formation of the SAM. We have isolated the putative *SHO1* maize orthologue, that exhibits a significant sequence similarity. The correspondence between *sml* and SHO1 will be revealed by polymorphisms detection and association studies.

The morphological and histological analysis of the dgr phenotype reveals a variety of plant abnormalities affecting different plant organ, including leaf, inflorescence, root and stem; the severity of defects may vary widely within a single mutant plant. Recessive mutations in the maize *leafbladeless1* (*lbl1*) gene lead to a very similar phenotype.

It is remarkable that both *SHO1* in rice and *lbl1* in maize are involved in the biosynthesis of the ta-siRNA, suggesting that a small RNA-mediated gene regulation operates at the critical step of SAM formation and maintenance in rice and maize. The analysis of the double mutant *sml-lbl1* will define the relationship between these two genes.

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Poster Abstract – D.41

LOCALIZATION AND FURTHER CHARACTERIZATION OF *UVR8*, A GENE INVOLVED IN *ARABIDOPSIS THALIANA* ROOT AND SHOOT DEVELOPMENT

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uvr8 gene, root and shoot development, Arabidopsis thaliana

The *uvr8* gene was identified in our laboratory as able to restore growth of the osmotic unstable yeast mutant *mpk1-/ppz1-* in absence of sorbitol. Gain and loss of functions in *A. thaliana* plants revealed that, besides the plant's response to UVB (Kliebenstein et al, 2002, Plant Physiol 130: 234-243) the gene controls plant development under non UVB-stressing conditions.

Root length and the number of lateral roots were significantly reduced in the *uvr8* over-expressing lines, while an opposite effect was observed in antisense lines and in a knock-out mutan from the Salk collections. Consistent with these data, hypocotyl elongation was inhibited in *uvr8* overexpressing arabidopsis seedlings. Cotyledon expansion was not affected in these seedlings, though petiole length was visibly reduced. In contrast, antisense seedlings displayed an increase in cotyledon size, while hypocotyl elongation was not significantly affected compared to control seedlings. Negative effects of *uvr8* on hypocotyls and cotyledon expansion were further confirmed in an *uvr8* knock-out mutant.

Experimental data with the GUS-fused promoter and the YFP::UVR8 protein evidenced a complex pattern of expression. The gene was primarily expressed in the L1 layer of meristematic shoot apex and in hypocotyls and cotyledon epidermis. Cellular localization of UVR8-YFP revealed that the UVR8-YFP signal was localized in the root columella cells as well as in the stele and lateral root cap. This localization pattern of the *uvr8* gene and protein suggests a link with auxin transport and its redistribution inside the plants. Since the content of flavonoids was higher in over-expressing *uvr8* plants, through the transcriptional regulation of the CHS gene, we speculate that the observed effects on plant root and shoot development might be due to the interference of these secondary metabolites with auxin transport and distribution.

We acknowledge the technical and the inspiring support of Drs. Massimiliano Sassi and Ida Ruberti (IBPM, CNR, Rome), in the confocal analysis, visualization of GUS activity and phenotypic analysis.

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Poster Abstract – D.42

THE *ARABIDOPSIS* MOB1-LIKE GENE IS INVOLVED IN BOTH VEGETATIVE GROWTH AND REPRODUCTIVE BEHAVIOUR

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plant development and reproduction, Arabidopsis thaliana, gametogenesis, seed set

The MOB family includes a group of cell cycle-associated, non-catalytic proteins highly conserved in eukaryotes, whose founding members are implicated in mitotic exit and co-ordination of cell cycle progression with cell polarity and morphogenesis. Two distinct Mob proteins, Mob1 and Mob2, are known in fungi, while an expansion in metazoans gives rise to six in human, four in *Drosophila*, and four in *C. elegans*. For what concerning plants, alfalfa Mob1-like genes were shown to be specifically expressed in degenerating megaspores of normal ovules and in enlarged megaspore mother cells and embryo sacs of apomeiotic ovules. Gene products were also found in microspore tetrads at the beginning of pollen development as well as in tapetum cells of anthers undergoing programmed cell death to allow pollen dispersal at maturity. Present research deals with the elucidation of the role of Mob1-like genes in order to gain further insights on their function in plants. Functional analysis of Mob1 genes of *Arabidopsis thaliana* (loci At5g45550 and At4g19050) was attempted by using RNA-interfered Mob1 mutants. Silenced single-insertion homozygous lines were investigated on the basis of plant morphological traits and cytohistological observations of female meiosis and gametogenesis. Both temporal and spatial gene expression patterns of AtMob1-like genes were also analyzed by means of Real-Time PCR with member-specific primers and immuno-localization within ovules using polyclonal antibodies against MOB1 proteins.

Analysis of gene expression in the plant organs revealed the presence of Mob1-specific transcripts in all analyzed samples, even if a stronger expression was detected in flowers and siliques. It is worth mentioning that AtRNAi lines were characterized by a marked decrease of Mob1 gene expression within flowers at different developmental stages. On the whole, our data support an altered growth habit and a strongly reduced seed set in the Mob1-interfered plants. In particular, a faster development of plants along with thinner shoots and smaller flowers and siliques were observed. Moreover ovules were shown to contain binucleated megaspores and non-polarized embryo sacs. To confirm the possibility of those unreduced megaspores to proceed throughout gametogenesis, leading to the formation of unreduced functional egg cells, a FCSS analysis of

RNAi lines was attempted. Similarly, image densitometry and pollen cytometry was performed to assay possible variation for the ploidy of pollen grains. Sub-cellular localization of MOB1 proteins within somatic and reproductive organs was also attempted by means of Arabidopsis lines characterized for the production of the GFP::MOB1 fusion protein.

Overall results in terms of gamete ploidy along with transcript expression and protein localization patterns in the Arabidopsis RNAi Mob1 lines are reported and critically discussed.

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Poster Abstract – D.43

IDENTIFICATION OF *ACL5* AS A TARGET OF HD-ZIP III TRANSCRIPTION FACTORS

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HD-ZIP III transcription factors, spermine, target genes, vascular development

The *Arabidopsis* HD-ZIP III transcription factor (TF) family consists of five highly related proteins involved in several developmental processes including vascular development. To gain more insights on the role of the HD-ZIP III TFs in the regulation of plant development, we searched for their target genes. A screening of the *Arabidopsis* genome database identified 390 genes containing the BS-III element, a 11 bp pseudo-palindromic sequence recognized by the HD-ZIP III proteins *in vitro*. Among these genes, *ACAULIS5* (*ACL5*) encoding a spermine synthase has been chosen for further investigation based on its putative regulatory role in vascular development. The loss-of-function *acl5-1* mutant is dwarf and characterized by the formation of an increased number of veins and vascular elements in leaves and stems (Hanzawa et al., 1997, 2000; Clay et al., 2005). Further phenotypic analysis of *acl5-1* revealed that it produces more lateral root primordia than wild-type plants, indicating a negative role of spermine or related molecules in this process.

As a first step to investigate whether *ACL5* is indeed a target of HD-Zip III TFs, *in vitro* DNA binding assays have been performed. EMSA experiments demonstrated that the HD-Zip III domain specifically recognizes an *ACL5* promoter region comprising the BS-III element but not a derivative region carrying mutations in BS-III. Next, to investigate whether one or more HD-Zip III TF regulates *ACL5* expression through the BS-III element *in vivo*, transgenic plants expressing the GUS reporter gene under the control of either the *ACL5* promoter or a derivative mutated in the BS-III element have been generated and characterized. Histochemical analysis revealed that an intact BS-III element is essential for GUS expression at the very early phases of vascular development in all the organs examined (leaf primordia, primary root an lateral roots). To assess the relevance of spermine production in procambial cells for proper vascular development and lateral root formation, the *acl5-1* mutant was complemented with the *ACL5* gene driven by its own promoter or a derivative promoter mutated in the BS-III element. The wild-type construct rescued all aspects of the *acl5* mutant phenotype. In contrast, plants expressing *ACL5* under the mutated promoter retain some aspects of the *acl5* phenotype such as increased number of veins in leaves and more lateral roots. Together the data demonstrate that *ACL5* expression and thus spermine synthesis is regulated through the action of one or more HD-Zip III TF at the early stages of vascular development and lateral root formation.

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Poster Abstract – D.44

EXPANSIN: EXPRESSION PATTERN, SUBCELLULAR LOCALIZATION AND RECOMBINANT PROTEIN PRODUCTION

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expansin gene, GUS expression, cell wall, Petunia hybrida

Expansins are the most important cell wall proteins involved in the regulation of cell wall enlargement in growing cells, acting in pH-dependent manner (McQueen-Mason et al., 1992; Cosgrove 2000; Lee et al., 2001). Gene-expression studies have shown that many expansin genes are expressed in a pattern that is consistent with their involvement in growth of specific organs or cell type. To identify the promoter sequence of *PhEXP1A*, a *P. hybrida W138* dTph1 insertion library has been screened and we isolated 1008 bp upstream of *PhEXP1A* coding sequence. Analysis of the *PhEXP1A* promoter sequence revealed the existence of several *cis*-acting transcriptional factors characteristic of expansin genes. To investigate the spatial and temporal expression of *PhEXP1A* during plant development, we constructed transgenic *P. hybrida* plants harboring a recombinant reporter gene (GUS) under *PhEXP1A* promoter sequence control. Furthermore, to examine whether PhEXP1A is capable of translocanting in the cell wall, plants of *Petunia* were transformed with a DNA construct encoding eGFP-EXP1A fusion proteins in C-terminal region (*PhEXP1A:GFP*) under the *CaMV 35S* promoter and examined their subcellular localization.

The production of active recombinant expansins A has proven largely unsuccessful and the crystal structure is still undefined. Their mechanism of action, not defined yet, appears to involve the disruption of hydrogen bonds between cellulose microfibrils and cross-linking glycans predisposing cellulose sling and consequently cell expansion. Another aim of this work is the production and purification of recombinant PhEXP1A, a first expansin A isolated in *Petunia hybrida*. Here, we report the construction and the expression of recombinant expansinA from *Petunia hybrida* in a bacterial expression system. The coding sequence of *PhEXP1A* was introduced into pDEST17 bacterial expression vector, using Gateway® Technology and highly expressed in derivatives C41(DE3) of the *Escherichia coli* strain BL21(DE3).

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Poster Abstract – D.45

ANALYSIS OF THE *FUSED LEAVES* (*FDL*) MUTANT AS A MEAN TO STUDY COLEOPTILE AND CUTICLE ORGANIZATION DURING MAIZE EMBRYO AND SEEDLING DEVELOPMENT

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programmed cell death, embryo development, cuticle

The isolation of genes affecting shoot formation is an important prerequisite for understanding the logic of plant development as well as for manipulating plant architecture. To this aim, maize transpositional mutagenesis has been adopted in our laboratory and has led to the isolation of developmental mutants affecting shoot organization. One of them, the *fused leaves* (*fdl*) mutant, will hereby be described. The *fdl* mutant was identified in an active *Suppressor-mutator* (*Spm*) line. Its phenotype is linked to an *Spm* insertion in a genomic region with homology to a Myb R2R3 motif.

Mutant *fdl* seedlings exhibit distinct features, such as a thicker and shorter coleoptile whose opening is delayed and occurs with an irregular lateral fracture in contrast to the clear-cut hole that is formed in the wild-type. In addition, during *fdl* seedling growth, regions of adhesion between the coleoptile and the first leaf or, alternatively, between the first and the second leaf are observed. Seedling defects can be traced back to earlier events that take place prior to germination.

Mutant and wild-type embryos have been compared for the occurrence of the programmed cell death (PCD) process during different stages of development. Results obtained with the TUNEL method demonstrate that in wild-type embryos both the scutellum and the coleoptile undergo PCD. By contrast, in the mutant embryos the TUNEL positive signals were not detected in the coleoptile primordium, although they were visible in the scutellum. It is thus conceivable that a specific set of genes involved in the PCD is activated in the normal coleoptile at a defined developmental point. The coleoptile, as well as other embryonic organs that have a transient function, is therefore "prepared" to die during embryogenesis.

Mutant seedling epidermal cells have been studied by means of transmission electron microscopy. The presence of fusions implies the absence of the cuticle between the two adherent epidermis surfaces. In contrast, the free surface of the leaves exhibits a substantial layer of cuticle. Moreover, in the fused regions, the two cell walls appear as a single structure so that the cell walls of the organs involved are not morphologically distinguishable. A defective distribution of epicuticular waxes, with bare zones scattered among covered regions, was also shown by the scanning electron microscope and the environmental scanning electron microscope analysis on the free surfaces of the first and second mutant leaf. All these defects are recovered at the third leaves stage.

The characterization of this pleiotropic mutant allowed us to gain more insight into two important aspects related with the early phases of maize plant development. The first one is related to the coleoptile, which is the first organ emerging from the seed whose function is to protect the

young emerging shoot. The establishment of a PCD program in this organ prior to germination is necessary for its correct opening during seedling growth. We have also found that correct cuticle organization along with epicuticular waxes deposition are required during late embryogenesis and seedling growth, not only for the establishment of an appropriate barrier against environmental stress, but also for determining organ separation.

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Poster Abstract – D.46

TRANSCRIPTOME ANALYSIS OF LEAF SENESCENCE IN *MEDICAGO TRUNCATULA*

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Medicago truncatula, senescence, cDNA-AFLP, gene ontology, nitrogen mobilization

Senescence studies are important in legumes, where key events of the process are a massive degradation of proteins and a consequent reallocation of nitrogen to grains. *M. truncatula* is a model organism for legumes (its genome is undergoing sequencing, large collections of ESTs are available) and information obtained in this species is likely to be easily transferred to agronomically important crops.

The cDNA-AFLP technique was used to identify more than 500 genes, which were cloned and sorted into functional categories according to their Gene Ontology annotation. Comparison with the *Arabidopsis* leaf senescence reveals that the process is roughly conserved. However, important differences exist in some of the transcription factors involved and in the mechanisms of amine mobilization, which might reflect the uniqueness of nitrogen biology in legumes. In addition, we have observed that a minority of the genes regulated during leaf senescence are equally involved in nodule senescence or nitric oxide treatment; nevertheless, a large part of these genes have regulative functions, suggesting a conserved mechanism in orchestrating the different processes.

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Poster Abstract – D.47

SEEKING FOR THE *PIN* GENE FAMILY OF AUXIN EFFLUX CARRIERS IN *ZEA MAYS*: A MULTIPLE APPROACH

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auxin, PIN, polar auxin transport, Zea mays

After the formulation of the chemiosmotic hypothesis for auxin transport, genetic approaches allowed the identification of genes involved in auxin polar transport in *Arabidopsis thaliana*. Because the chemical properties of IAA suggested that auxin efflux is the limiting step, the isolation of auxin efflux carriers became the main objective of scientists. Molecular characterization of the *pin1* mutant let the identification of the first member of PIN-FORMED gene family that encode transmembrane proteins with a similarity to a group of bacterial transporters. Subsequently, seven other genes similar to *PIN1* were found in Arabidopsis genome and PIN proteins have been shown to play a rate-limiting role in the catalysis of efflux of auxin from cells. PIN proteins asymmetrical cellular localization determines the direction of cell-to-cell auxin flow, creating auxin gradients that regulate a wide variety of processes, including embryogenesis, all type of organogenesis, vascular tissue differentiation, root meristem maintenance, root elongation, apical dominance and tropic growth responses to environmental stimuli.

Genes homologous to the Arabidopsis *PIN* are present in genomes throughout the plant kingdom, from the model moss *Physcomitrella patens* to all vascular plants and the relatively high amino acid identity between PIN proteins suggests that all the *PIN* genes diverged from a single ancestral sequences. Phylogenetic analysis of PIN sequences from *Oryza sativa* and *Triticum aestivum* revealed that the monocot *PIN* family is wider and divergent than dicots one, with two or three genes homologous to one Arabidopsis *PIN* gene. Wheat and rice present respectively three and two closely related *PIN1* genes. On the other hand TaPIN9 and OsPIN9 do not clusterize with any dicot sequence, suggesting the presence of at least one monocot-specific PIN protein.

Zea mays presents an even wider and more divergent *PIN* family if compared with the wheat and rice ones. We identified three orthologs of *AtPIN1*, called *ZmPIN1a*, *ZmPIN1b* and *ZmPIN1c* and we mapped them respectively on the chromosomes 9, 5 and 4. Our aim is to identify maize *pin1* mutations by the genetic and molecular characterization of spontaneous mutants mapped in proximity of the *ZmPIN1* gene positions. At the same time, we are complementing the Arabidopsis *pin1* mutant with *ZmPIN1* cDNA full length sequences to asses if they really act as auxin efflux carrier.

Moreover, the widening of maize *PIN* family was confirmed by the cloning of two genes closely related to *AtPIN2*, and by the identification of the putative orthologs to *AtPIN3*, *AtPIN4* and *AtPIN6*. Currently, we are analyzing their expression patterns by semiquantitative RT-PCR and by *in situ* hybridization.

In addition RT-PCR experiments allow us to identify splicing variants for *ZmPIN1b*, *ZmPIN1c*, *ZmPIN2* and *ZmPIN3*. Their role in maize development is still under analysis.

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Poster Abstract – D.48

EXPRESSION ANALYSIS AND FUNCTIONAL CHARACTERIZATION OF TWO *NF-Y* GENES IN *ARABIDOPSIS THALIANA*

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NF-Y genes, Arabidopsis thaliana, expression analysis, pollen development, aborted seeds

In a statistical analysis of over 500 eukaryotic promoters, including many from plant species, the CCAAT box was one of the most ubiquitous elements, being present in 30% of them. In yeast and mammals, the CCAAT box is recognized by NF-Y, a trimer composed of distinct subunits: NF-YA, NF-YB and NF-YC, all required for DNA-binding. The NF-YB and NF-YC subunits form a tight dimer, via protein structures similar to the Histone Fold Motif -HFM- a conserved protein-protein and DNA-binding interaction module. Heterodimerization results in a surface for NF-YA association and the trimer can then bind to DNA with high specificity and affinity.

In *Arabidopsis thaliana*, the complete NF-Y family is composed of 29 genes: 10 *NF-YAs*, 10 *NF-YBs* and 9 *NF-YCs*. We studied two *NF-Y* genes of the *AtNF-YA* sub-family, which are very close in the phylogenetic tree and both expressed in vegetative and reproductive tissues. Our aim was the characterization of their functional role.

Detailed expression analysis revealed that they are expressed during ovule and pollen development and have a similar expression pattern. In one single mutant, pollen development is damaged, in since some pollen grains at the mature stage are defective; instead the other single mutant does not show any phenotypic defect.

In order to verify the functional relationship between these genes, we decided to analyze the double mutants. These are lethal and moreover the segregation analysis of plants homozygous for one gene and heterozygous for the other gene revealed that the ratio of normal and defective seeds (aborted seeds and arrested embryos) is 1:1, suggesting that there is a gametophytic lethality.

Morphological characterization of these plants showed that there is male lethality: in fact the ovule development is normal and the pollen development at early stages is the same to wild-type, but a lot of mature pollen grains are defective.

These data indicate that these *AtNF-YA* genes have a role during pollen development and that probably they are partially redundant. Reciprocal crosses and segregation analysis will confirm the male lethality and further analysis will show what determines the pollen development arrest.

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Poster Abstract – D.49

FLAVONOID METABOLISM IN *MEDICAGO TRUNCATULA* MUTANTS

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Barrel medic, anthocyanin biosynthesis, gene expression, metabolism

Barrel medic (*Medicago truncatula* Gaertn.) is a self-fertile, annual, and diploid plant that has been selected as a model legume. Flavonoids are plant antioxidants synthesized by the phenylpropanoid metabolic pathway and their benefits for plant, human and animal health are known. In this work six mutants of barrel medic affected in flavonoids biosynthesis have been characterized. These mutants, obtained by chemical (tilling) or physical (fast-neutron radiation) mutagenesis, showed either an altered pattern or an absence of pigmentation in leaves and flowers compared to wild-type plants. A strong reduction of the total amount of anthocyanins present into mutant leaves was also found. At metabolite level, the amount of flavones measured with LC-MS was also affected by the mutations. The tricin-3GluAc was the most accumulated product in mutant leaves compared to apigenin-3GluAc in wild-type. We analysed the expression of structural genes and selected transcriptional factors (*Myb*, *Myc* and *MADS*-box genes, WD40 protein) involved in flavonoids biosynthesis by RT-PCR and qPCR and we measured altered expression profiles in the mutant compared to wild-type leaves. For instance, when the amount of anthocyanins was very low, the glutathione S-transferase (GST) expression was strongly reduced; one mutant showed a complete suppression of the UDP-glucose:anthocyanin 5-O-glucosyltransferase (5GT) expression (the last enzyme of anthocyanin biosynthesis). We observed a correlation between the amount of anthocyanins and the expression of PAP1, a specific *myb* gene related to anthocyanin synthesis.

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Poster Abstract – D.50

TRANSCRIPTONIC ANALYSIS OF KERNEL GROWN AND DEVELOPMENT

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high-throughput experiments, endosperm mutants, transcriptomics, gene expression

In maize, the zein synthesizing system is particularly adapted for the study of the regulating mechanisms of plant genes because i) its expression is restricted to a specific tissue and stage during seed development and ii) of the availability of mutants useful in dissecting the regulatory processes taking place in the developing seed. Studies on genetic mutations that affect the accumulation of different zeins have demonstrated the existence of several regulatory signals controlling the expression of specific members of the zein family which confer an opaque phenotype to the endosperm. For example, the recessive mutations *opaque2 (o2) and opaque7(o7)* induces a specific decrease in accumulation of 22 and 19-kD alpha-zeins, respectively, while the *opaque15 (o15)* mutation exerts its effect primarily on the 27-kD gamma zeins. The recessive mutation *opaque6 (o6)* and the dominant or semi-dominant mutations *Floury (Fl2*), *Defective endosperm *B30 (De*B30*), and *Mucronate (M*c) cause a more general reduction in accumulation of all zein classes. In recent years, the development of extensive maize cDNA libraries, along with computer software to systematically characterize them, has made it possible to analyze gene expression in developing maize endosperm more thoroughly. Accordingly, we have used cDNA microarray technology to investigate the transcription profiles and differential gene expression of maize endosperm from two *opaque* mutants (*o2* and *o7*) and the double mutant combination (*o2o7*). Microarray slides containing the entire Zeastar unigene set were hybridized with probes derived from endosperm tissue harvested 15 days after pollination (DAP) and derived from the A69Y*wt*, A69Y*o2*, A69Y*o7*, and A69Y*o2o7* isogenic lines. All microarray experiments were performed in triplicate using dye swapping, hence giving rise to 12 independent measurements for each EST, considering the presence of duplicate spots on each slide. Ratios between wild type and mutant expression levels were calculated and ESTs exhibiting ratios below 0.5 or over 2 were selected for further analysis. The results clearly showed the prevalence of genes showing distinct expression patterns in the A69Y*wt* and A69Y*o2* genotypes. Conversely, the A69Y*wt* and A69Y*o7* genotypes show less evident differences in expression levels. The A69Y*o2o7* double mutant exhibits differences in expression patterns resembling those obtained for the A69Y*o2* genotype. A plot of A69Y*o2* vs. A69Y*o7* expression levels showed the cumulative effect of both genotypes revealing a high number of genes with distinct expression patterns. Among the ESTs considered, 17,1% exhibited a down-regulated expression profile. The *o2* mutation was associated with 649 down regulated ESTs, 508 down-regulated ESTs were identified in A69Y*o7* background, whereas 759 ESTs showed a reduced expression pattern in A69Y*o2o7*. Up-regulated expression profiles were found for 3.23% of the ESTs considered. One hundred and thirteen up-regulated ESTs were identified in the A69Y*o2*, 26 in the A69Y*o7*, and 86 in an A69Y*o2o7* backgrounds, respectively. Among the ESTs identified, 36.7% exhibited relevant homology with sequences deposited in public databases and were

univocally associated with known biological processes related to amino acid and carbohydrate metabolism, signal transduction, protein turnover, transport, and protein folding. In addition, 3 transcription factors different from *O2* appear down-regulated. Collectively, the results may provide a framework for investigating a common mechanism that underlines the *o2* and *o7* kernel phenotypes.

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Poster Abstract – D.51

TOWARD MAP-BASED CLONING OF THE *REDUCED GRAIN FILLING* 1 GENE IN MAIZE

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fine mapping, Bulked segragant analysis, recombinant pools, reduced grain filling (rgf1)

The grain filling is an important trait associated with the yield of crop. The reduced grain filling (*rgf*1) phenotype in maize is caused by a dominant mutation and presents final grain weight reduced to 30% of that of the wild type. Its pericarp is partially unfilled, while the embryo and somatic tissues are viable and indistinguishable from wild type. Respect to other small size seed mutants, *rgf*1 shows dosage-dependent reduction. This aspect makes *rgf*1 a good candidate for understanding endosperm development and seed filling. Using back-cross populations *rgf*1 was mapped to bin 2.04 of chromosome 2 between two SSR markers, bnlg1613 and bnlg1140, at a distance of 4.4cM. The region between the 2 markers was enriched using interval mapping pools: the mapping interval was reduced to 0.5cM with the aid of AFLP markers. These results were confirmed by testing an F3 population consisting of 1406 individuals obtained from the cross B37 x *rgf*1. Two single copy AFLP markers co-segregated with the mutant locus and were used as probes for screening a BAC library. Additional *rgf*1 alleles were generated by transposon insertion. Out of 650,000 F1 seeds obtained from the cross of *rgf*1/*rgf*1, *Mu* to wild type, 6 independent insertions were isolated. Genetic and molecular evidences are reported to sustain the recessive, wild type and lethal nature of the new alleles.

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Poster Abstract – D.52

APOMIXIS IN ST. JOHN'S WORT (*HYPERICUM PERFORATUM* L.): AN OVERVIEW ON RECENT FINDINGS

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apospory, megagametogenesis, candidate genes

In angiosperms, sexual reproduction is characterized by an alteration between clearly defined sporophytic and gametophytic generations. Differently from the common sexual behaviour, apomixis defines alternative reproductive strategies in which seeds are generated asexually without either meiotic reduction and fertilization in ovules. Apospory belongs the apomictic reproductive strategies and it is characterized by the development of one or more functional embryo sacs from somatic nucellar cells, known as the aposporous initial cells. In such a reproductive system, the unreduced egg cell develops parthenogenetically into an embryo and the endosperm can develop either autonomously or by fertilization dependent way (pseudogamy).

Recent studies suggest the adoption of *Hypericum perforatum* L. as a model species for this variant of gametophytic apomixis.

Great strengths aimed at elucidating the cytological and molecular basis of plant sporogenesis and gametogenesis have been performed and a number of mutants lacking various components of the embryo sac development have been identified in different model sexual species (*e.g.*, Arabidopsis and corn). Even so, a few data are available for the aposporous development in model apomicts, including *Hypericum perforatum*. Advances towards the comprehension of the aposporous apomictic pathway have been recently obtained following a two-steps approach: i) definition of the developmental window in which megasporogenesis and megagametogenesis occur as well as the aposporic initials take place into ovules; ii) cloning and studying the expression patterns of key genes, candidate to be part of the aposporic molecular machinery, during different developmental stages of ovules.

With respect to the cytohistological analyses, flowers from plants of known sexual and aposporic behaviour were harvested at different developmental stages and both sporogenesis and gametogenesis investigated by means of ovule whole mount microscopical observations. Sexual development pathways were studied and major recognisable steps related to morphological traits of flowers. The illegitimate reproductive pathway represented by Aposporous initials (AI) establishment and development were characterized and integrated within the reproductive model.

Concerning the molecular approach, three different genes: HpEMB2733-like, HpARIADNE and HpAPOSTART, were selected as candidates, cloned and the expression patterns assayed in reproductive organs. More in detail, EMB2733-like as well as APOSTART were previously found to be differentially expressed between aposporic and meiotic reproductive organs of *H. perforatum* and *Poa pratensis*, respectively. Similarly, a CAPS marker designed on a RING-finger gene (*i.e.*, HpARIADNE), was previously found to be in strong Linkage Disequilibrium with the apomixis trait and therefore selected for further investigations.

Overall results of ovule cytohistological investigations along with gene expression analyses are presented and critically discussed.

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Poster Abstract – D.53

ISOLATION AND CHARACTERIZATION OF GENOMIC REGIONS CONTAINING APOSTART IN *POA PRATENSIS* L.

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APOSTART, apomixis, meiosis, genomic libraries, promoter

Poa pratensis L. is a cool-season grass of great importance for forage and turf production in the temperate climates of the world besides it is important for the production of high quality amenity and sport lawns. This species reproduces facultatively through aposporous apomixis and sexual outcrossing. In natural populations plants showing a wide range of combinations of sexuality and apomixis have been found, including completely sexual, intermediate apomictic and nearly obligate apomictic genotypes.

For understanding the molecular genetics of complex traits such as apomixis, the isolation of specific genes is crucial. With this respect, the use of new cloning strategies could provide innovative tools to isolate genes involved in traits of interest. The choice of the right method is related to the kind of information desired. We have demonstrated that a cDNA-AFLP strategy, applied to developmental staged inflorescences, was useful to identify several ESTs differentially expressed between apomictic and sexual genotypes of *P. pratensis*. In particular, by using this strategy, we have isolated a gene which we termed APOSTART and which shows both a different expression between apomictic and sexual genotypes and a flower-specific localization.

For these reasons we have created 3 genomic libraries using 2 apomictic and 1 sexual genotypes with three aims: i) to isolate DNA regions containing APOSTART members/alleles; ii) to identify genes linked to APOSTART and iii) to sequence regions upstream APOSTART to verify the presence of promoter, 5'-UTR and of possible enhancers.

Here we describe the screening of these genomic libraries for clones carrying the APOSTART gene and the full-length clone sequencing. The bioinformatic characterization of these clones is reported and discussed.

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Poster Abstract – D.54

LINKAGE MAPPING OF CANDIDATE GENES FOR APOMIXIS

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apomixis, linkage mapping, mode of reproduction, gene mapping

Seed is one of the key factors of crop productivity and the comprehension of the mechanisms underlying seed formation is crucial for the quantitative and qualitative progress of agricultural production. In angiosperms two pathways of reproduction through seed exist: sexual, or amphimictic, and asexual, or apomictic. Genetic linkage mapping of apomixis loci in natural apomicts is one of the possible approaches to isolate genes related with the trait and transfer apomixis into crop species. The overall results indicated simple, dominant inheritance either of apomixis as a whole or as a few independent loci. Nevertheless in an increasingly number of species apomeiosis, either apospory or diplospory, and parthenogenesis have recently been found inherited independently. Up to now, no evidence for recombination suppression was found at the locus for parthenogenesis in species such as *Erigeron annuus* and *Poa pratensis* for which mapping data were available. *P. pratensis* (Kentucky bluegrass) is a hardy, persistent, attractive forage and turf grass adapted to a wide range of soils and climates. Its mode of reproduction is extremely variable and can range naturally from nearly obligate apomixis to complete sexuality.

The main goal of the study was to map selected genes (APOSTART, BABY BOOM, PpSERK and PpMET) as well as some 179 ESTs, differentially expressed between sexual and apomictic genotype of *P. pratensis* into a genetic linkage map. By crossing a completely sexual clone (S1/1–7) with a highly apomictic genotype (L4) we developed a segregating population which was characterized for its mode of reproduction. The linkage analysis was carried out following a pseudo-testcross strategy and using molecular data from 88 genotypes of the F1 population. We have applied SAMPL, AFLP and MFDP techniques to generate the framework for mapping the candidate genes and the putative loci for apospory and parthenogenesis with the aim of looking for those co-segregating with the mode of reproduction (apospory/parthenogenesis).

The current map for S1-1/7 covers about 1700 cM and consist of 270 markers distributed in 26 linkage groups, considering just those groups containing EST-derived markers. In the L4 map, 200 markers are distributed in 25 linkage groups for a total of 1400 cM covered.

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Poster Abstract – D.55

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF MEMBERS OF THE ETHYLENE RESPONSIVE FACTORS FAMILY DURING THE GRAPEVINE BERRY RIPENING

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Vitis vinifera, berry ripening, ethylene responsive factors

There are compelling evidences of the role played by ethylene in the ripening process of the grapevine berry. An endogenous ethylene peak has been observed just before véraison time and treatments with exogenous ethylene were shown to influence the onset of ripening and of the related processes such as the anthocyanins accumulation.

We studied the gene expression of four grape ethylene responsive factors (ERFs) and one ethylene receptor, to characterize their role during berry ripening. Two ERF genes appeared to be transcribed in a berry-specific manner, one (VvERF1) being preferentially expressed in the skin and the pulp, the other (VvERF3) being highly expressed in the seed. Their transcriptional profiles along berry development were also quite different. The ERF1 transcript concentration increased upon véraison and remained high during ripening, whereas ERF3 transcript concentration decreased after véraison.

The study of the ERF1 profile in early and late cultivars confirmed the strict association between the onset of ripening and the induction of its transcription.

ERF transcription factors are known to regulate the transcription of their target genes by binding to the GCC box present in the promoter sequence.

Data of ERF1 trans-activation using a tobacco transient expression system to discriminate possible target genes, will also be presented.

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Poster Abstract – D.56

IN SILICO CHARACTERIZATION AND EXPRESSION ANALYSIS OF CLASS 1 DXS, A CANDIDATE GENE FOR MONOTERPENE ACCUMULATION IN GRAPE BERRIES

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DXS, aroma, HRGC-MS metabolic profile, grape

In *Vitis vinifera* monoterpenoids, sesquiterpenoids and C13-norisoprenoids are among the most important fruit aroma and flavour components and give a significant contribute to the quality of both table grapes and wines. Plant-derived volatile terpenoid compounds occurring in wines are mainly stored as non-volatile, water-soluble glycoside derivatives in exocarp cell vacuoles, although some terpenoids may also be present as free volatiles. Two independent pathways, the cytosolic mevalonate (MVA) and the plastidial mevalonate-independent 1-deoxy-D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate (DOXP/MEP) pathway, form the C5-units IPP (isopentenyl diphosphate) and DMAPP (dimethylallyl diphosphate), the initial substrates for the biosynthesis of the ca 22.000 different terpenes known in higher plants.

From two mapping experiments in grape, based on Italia x Big Perlon and Moscato bianco x V. riparia segregating populations, we found that class 1 *dxs* gene encoding 1-deoxy-D-xylulose 5-phosphate synthase, the first committed enzyme of the DOXP/MEP pathway, co-localized with a stable major QTL for berry content of monoterpenes linalool, geraniol and nerol. Sequence alignment showed that VvDXS1 had high homology to known DXS proteins from other plant species and contained the conserved N-terminal plastid transit peptide. Phylogenetic analysis confirmed that VvDXS1 belonged to the plant DXS1 cluster.

Here we present 1) the first *in silico* analysis of grape DXS genes based on the whole genomic sequence and 2) the first investigation of the relationship existing between VvDXS1 mRNA expression level and monoterpenoids accumulation as detected by HRGC-MS (high resolution gas chromatography-mass spectrometry) analysis of berries sampled from pre-veraison to over-ripening, in aromatic and non-aromatic grapevine varieties.

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Poster Abstract – D.57

VARIATION OF METABOLIC PROFILES IN DEVELOPING MAIZE KERNELS UP-AND-DOWN-REGULATED FOR THE *HDA101* GENE

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metabolic variation, gene regulation, chromatin remodelling, gene silencing

To shed light on the specific contribution of the histone deacetylases101 (HDA101) in modulating metabolic pathways in the maize seed, we have investigated changes in the metabolic profiles of kernels obtained from *hda101* mutant plants. In the field of metabolomics the analysis of metabolic changes in time is a fundamental aspect of understanding the biochemical response of an organism to an external perturbation (Lindon et al., 2001). As processes develop through time, the metabolic responses also exhibit dynamic variation. Therefore, monitoring these changes results in characteristic patterns for each type of perturbation. Principal component trajectories have been constructed from Nuclear Magnetic Resonance (NMR) data to investigate the changing multivariate biochemical profile during development of a toxic lesion (Keun et al., 2004). However, this kind of analysis, although effective for trajectory analysis, is not suitable to the simultaneous comparison of several parallel systems, and thus the use of alternate multi-way tools for optimally extracting metabolic trajectory and biomarker information have been investigated (Antti et al., 2002; Dyrby et al., 2005a). Multi-way analysis is the extension of the traditional multivariate analysis to array with more than two way, where data are characterised by several sets of variables that are measured in a cross fashion (Bro, 1997).

In this paper, we describe the application of multi-way Partial Least Square (N-PLS) to NMR spectra to evaluate the effect of the up- and down-regulation of HDA101 activity in terms of metabolites concentrations during maize seed development.

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Poster Abstract – D.58

IS EMPTY PERICARP4 INVOLVED IN NON-SEED TISSUES DEVELOPMENT?

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Zea mais, mitochondria, embryo rescue, pentatricopeptide repeats

Empty pericarp mutants represent a class of *dek* mutants with the most severe reduction in endosperm development. They are easily recognizable in segregating mature ears because they are devoid of endosperm material and flattened by compression from the surrounding normal seeds. To elucidate the molecular basis of these mutants a gene tagging approach was undertaken in our laboratory that lead to the isolation of the *empty pericarp4* gene.

The product of this gene belongs to the pentatricopeptides repeats (PPRs) family of proteins, one of the largest gene family in plant, so called because of the presence of a degenerated 35 aminoacid repeat, the PPR motif. About 80% of the PPR proteins are targeted in organelles (60% in mitochondria and 20% in chloroplasts), and it is generally assumed that PPRs play a role in controlling the organellar gene expression. Despite their great number in the plant genome many of the PPRs so far analyzed have a non-redundant function. The *empty pericarp4* (*emp4*) gene encodes a 614 amino acid protein containing nine PPR motifs, a signal peptide for mitochondrial import and two domains with unknown function, located at the C and at N-termini respectively. Functional analysis revealed that lesions in this PPR gene are associated with specific seed developmental defects, first recognizable in the basal endosperm transfer layer (BETL), a region located in the basal portion of the seed, characterized by the early presence of transfer cells that facilitate nutrients import into the maize kernel. Moreover the presence of a functional *emp4* in the maize kernel has been correlated with the accumulation of three mitochondrial transcripts, *rps2A, rps3* and *mttB.*

emp4 transcription occurs, even though at a very low level, in most plant tissues. Because of this observation we tried to estabilish if the *emp4* gene product, besides exerting a specific role during seed formation, is involved in other developmental events.

Homozygous *emp4* mutant embryos are retarded in their growth and unable to germinate. To analyze EMP4 function during postembryonic stages, immature embryos have been excised from the kernel and cultured on synthetic media. Following the embryo rescue approach we obtained homozygous mutant seedlings, whose genotype has been ascertained through a PCR based strategy. These seedlings exhibit delayed growth and are unable to reach reproductive maturity. Data will be presented on the morphological and molecular analysis of mutant roots, leaves and stem tissues. Changes in the mitochondrial and chloroplast population have been highlighted from the comparison of wild-type and mutant tissues by means of transmission electron microscopy. In addition, the expression of a sub set of mitochondrial genes has been investigated in the same

tissues by semi quantitative RT-PCR. The effect of the mutation on the expression of these genes in non seed tissues will be discussed.

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Poster Abstract – D.59

A MOLECULAR MODEL FOR EPIGENETIC MECHANISMS IN THE OPAQUE2-MEDIATED REGULATION OF GENE TRANSCRIPTION IN MAIZE ENDOSPERM

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epigenetic mechanisms, gene transcription, Zea mays

Several evidences in the past ten years have clearly demonstrated that epigenetic mechanisms, such as chromatin structure, histone modification, and cytosine methylation play a pivotal role in regulating gene transcription. These mechanisms are conserved in eukaryotes; however the peculiarities of plant development and of response to environmental cues result in a more flexible epigenetic regulation of the genome activity. This implies that plant-specificities in the epigenetic-mediated regulation exist. In addition, species-specific peculiarities, due to differences in genome organization, have also been reported. The precise mechanisms of the epigenetic-mediated regulation of gene transcription are beginning to be clarified in the Arabidopsis model plant. However, these mechanisms are again poorly understood in crop plants.

In this study we have employed the Opaque2 (O2) mediated control of gene transcription during endosperm development as a model system to investigate the epigenetic regulation in maize. O2 is a transcriptional activator belonging to the b-zip class, which has been extensively characterized in the past twenty years. O2 is specifically expressed throughout endosperm development and activates transcription of several target genes (e. g. 22 kDa α-zein, pyruvate orthophosphate dikinase1: PPDK1, lysine ketoglutarate reductase: LKR, etc.). Usually, O2 acts as a homodimer and binds a conserved O2-box within the promoter of its targets. Previous findings indicate that epigenetic mechanisms are involved in the O2-mediated transcriptional regulation, because cytosine methylation impairs the binding of O2 to O2-box and is related to uni-parental expression of 22 kDa α-zein in specific genetic backgrounds. To clarify the role of epigenetic mechanisms in the O2-mediated transcriptional regulation, we have characterized the epigenetic modifications of various O2 target genes in different developmental stages of endosperms and in sporophytic tissues, such as leaves, where O2 and most of its target are not expressed. First we have analyzed the chromatin structure using DNAse I accessibility assays. Subsequently, the cytosine methylation level and profile was assessed by means of restriction with the methylation sensitive enzyme McrBC and bisulfite sequencing, respectively. Chromatin immunoprecipitation (ChIP) technique with antibodies against specific post-translationally modified histones was employed to analyze the histone modification pattern and to investigate the timing of O2 and RNA Polymerase II binding to their target promoters. The results obtained in this study allow the formulation of a molecular model, which describes the role of epigenetic mechanisms in the regulation of transcription for the O2 target genes. This model implies three different transcriptionally states for the O2 target genes: silenced, poised, and activated state, each with a specific profile of epigenetic marks. The detailed results from this study will be illustrated and discussed.

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Poster Abstract – D.60

A LEAKY ALLELE OF *FALSIFLORA*, THE TOMATO ORTHOLOG OF *LEAFY*, PUTATIVELY UNDERLIES THE MUTATION *PISTILLATE*

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Falsiflora, flower development, flower induction, Solanum lycopersicum

The tomato recessive mutation *pistillate* (*pi*) directly recalls the phenotype of plants disrupted in the function of class B MADS-box genes, because it shows defects on the second and third floral whorl, mainly resulting in ectopic sepal and carpel features respectively. However, after we mapped *Pi* on the distal end of chromosome 3, the hypothesis of its allelism with class B genes was discarded, because none of the latter was compatible with the position of *Pi*. Alternatively, the signs of sepalization on the three inner floral whorls that are recoverable by electron scanning microscopy, coupled with the occurrence of a 'flower within flower' phenotype, suggested that a member of the *SEPALLATA* MADS-box gene family could be responsible for the *pi* phenotype. Again, the available map position of all the tomato members of the class E clade did not coincide with the mapping of *Pi*.

Differently, inside the genetic window containing *Pi* was located the marker *FA*, which turned out to represent *Falsiflora* (*Fa*), the tomato ortholog of the *Leafy* gene in *Arabidopsis* and *Floricaula* in *Antirrhinum*. Accordingly with the function of *Leafy*/*Floricaula* in inducing flowering and conferring flower identity to the meristem, *pi* mutant plants showed delayed flowering time, an increased sympodial segment and frequently a 'leafy' phenotype of the inflorescence. The class B-like phenotype shown by *pi* mutant plants is thus an indirect consequence of the mutation in *Fa* because *Fa*, as *Leafy*, is a positive regulator of class B MADS-box genes. Sequencing of the *Fa* genomic region in a wild-type and a *pi* plant evidenced a T to C transition in the first exon causing a methionine to threonine amino acidic substitution that disrupts a strongly conserved domain. Because *fa* mutant plants, as most of those mutated in orthologous genes in different species, generally do not form complete flowers, the *pi* mutation deserve a particular interest, representing a leaky *fa* phenotype witnessing the functional linkage between flower induction and flower organ identity specification.

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Poster Abstract – D.61

VOLATILE BENZENOID BIOSYNTHESIS IN *VITIS VINIFERA*

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Vitis vinifera, Petunia hybrida, benzenoid production

From the scent producing *P. hybrida* cv Mitchell was recently identified ODORANT1, an R2R3MYB-type transcription factor, which controls the synthesis of volatile benzenoids and regulates, at transcriptional level, shikimate pathway by the capacity to activate EPSPs promoter[1,2]

In this study we would like to identify genes involved in the synthesis of the principal volatile phenolic-benzenoids such as benzaldehyde (bitter almond taste in wine), phenylacetaldhyde, benzyll alcohol, 2-phenylethanol (rose) and vanilline (vanilla) that are found mainly in grape berry skin and that are involved in the primary aromas developing during berry ripening[3].

BlastP analyses were performed against the Genoscope Blast Server (www.genoscope.cns.fr) using the Petunia ODO1 sequence against the grapevine genome. [4].Three putative grapevine genes with the best sequences homology to PhODO1 were identified: VvODO1 (80%omology), VvODO2 (50% homology) and VvODO3 (56% homology) .

The level of the expression of each grapevine genes was analyzed in developing vegetative and reproductive organs of plants of *V. vinifera* cv. Corvina (clone 48) by real time RT-PCR experiments.

The transcriptional profile of these regulatory genes was also studied during development, maturation and withering of berries of *V. vinifera* cv. Corvina sampled in the season 2006.

VvODO1, VvODO2, VvODO3 were indepedently overexpressed in *P. hybrida* cv. Mitchell. Transgenic petunia plants and their flowers, expressing the heterologous genes, were analyzed for the expression levels of structural genes and their floral scent production.

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Poster Abstract – D.62

CLONING AND EXPRESSION OF *KALANCHOE XHOUGHTONII* KNOTTED-LIKE GENE (*KXHKN5*)

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In situ hybridization, Kalanchoe xhoughtonii, knox genes, RNA-interference, vegetative vivipary

Vegetative vivipary leads to formation of novel complete plantlets on mature organs. In *K. xhoughtonii* (n=51), a triploid interspecific hybrid between *K. daigremontiana* Hamet & Perrier (n=17) and *K. delagoensis* Ecklon & Zeyher (n=34), viviparous plantlets are formed on leaf margin notches in response to a long day photoperiod and their appearance follow a basipetal fashion. Several well known class 1 *knox* genes, as *Knotted1* (*Kn1*) from maize and *SHOOTMERISTEMLESS* (*STM*), *KNAT1* and *KNAT2* from *A. thaliana*, play an important role in meristem formation and maintenance. Several reports suggest that this class of homeotic genes could be involved in vivipary. In order to identify *knox* genes involved in vegetative vivipary in *K. xhoughtonii* hybrid, leaf tissue was collected before buds formation. Following RNA extraction and cDNA synthesis, semi-nested PCR was performed using anchored oligo-dT primer and degenerated primers designed on homeodomain sequence (Kobayashi et al., 2000). PCR products were cloned and sequenced. To identify full length coding sequence, nested 5' RACE was carried out using whole or digested cDNAs adaptor libraries. Four identified *knotted*-like genes belong to class 2 (*KxhKN1to KxhKN4*) and one to class 1 (*KxhKN5*) and their sequences were submitted to GenBank (NCBI), respectively: EU272787, EU272788, EU272789, EU272790, EU240661. Over-expression and silencing experiments of *KxhKN5* were performed in *K. xhoughtonii* and in eterologous systems, (*Osteospermum ecklonis*). To accomplish over-expression, the complete cDNA sequence of the gene (1161 bp), overdrive by 35S promoter and terminator, was cloned in the binary vector pGreen II (www.pgreen.ac.uk) that contain the NPTII gene, that confer resistance to kanamycin; the derived vector was transferred to *A. tumefaciens*. Post transcriptional gene silencing (PTGS) construct was prepared by cloning in pJM007 (Schattat et al., 2004), a 326 bp fragment of the gene in sense and antisense orientation in the specific cloning sites located at the left and at the right of the PIV2 intron. The silencing cassette was excided from pJM007 and cloned into the binary vector pGreen II NPTII. Following *A. tumefaciens* mediated genetic transformation with either over-expressing or silencing constructs and selection on medium containing kanamycin and cefotaxime to contain bacteria overgrowth, the regenerated shoots were isolated from the leaf explants and separately cultivated on propagation medium to establish plant clones. Some clones were acclimatized in greenhouse. To localize *KxhKN5* mRNA in early fase of *K. xhoughtonii* epiphylly, *in situ* hybridization was performed according to FISH Tag RNA Kit (Invitrogen, Carlsbad - California), with minor modifications.

In vivo, *K. xhoughtonii* plants over-espressing *KxhKN5* usually show a bushy phenotype with entire, rounded basal leaves and deeply palmated apical leaves. In control plants, leaves are lanceolate with dentated margin. Propagules formation is severely reduced in some transgenic plants. Transgenic *O. ecklonis* shows an assortment of phenotypes ranging from plants with small

leaves to plants with short internodes and bracts looking leaves. Extreme phenotypes survive only in vitro and resemble a cushion of dwarf bracteated stems.

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Poster Abstract – D.63

SCREENING OF A BAC LIBRARY OF *CITRUS SINENSIS* BY MEANS OF OVERGO PROBES FOR THE IDENTIFICATION OF CLONES CONTAINING GENE SEQUENCES WITH RELEVANT ROLES IN FRUIT QUALITY

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EST database, unigene, unique oligo, paralog, structural enzyme

Compared with the most commonly used gene-specific hybridization probes (subcloned DNA fragments, PCR amplimer products or DNA oligonucleotides), the overgo hybridization method (Ross et al., 1999) has several advantages (better hybridization kinetics, higher specificity labeling, low background of hybridization, low rate of false positives, high throughput, and ease of handling). The foundation of these advantages is the short sequence that is needed for an overgo probe, which increases the likelihood of finding suitable single copy regions from EST sequences or unigenes. Overgo probes are designed to 36/40-bp regions of cDNA that have been prescreened to mask out all known repeat elements. This assumes the availability of a comprehensive repeat element database that allows repeat masking of the target sequence. HarvEST:Citrus (http://harvest.ucr.edu/), an EST database-viewing software developed at the University of California – Riverside, displays 89 libraries and 229,570 ESTs from Citrus and Poncirus. We used the C38 assembly (36,980 unigenes) and the OligoSpawn software (http://138.23.191.145/) to design the overgo probes employed to screen a *Citrus sinensis* 'Vaniglia' BAC library. OligoSpawn software was used to select 22,444 "unique" oligos (36-bp oligonucleotides each of which appears in one unigene but does not occur, exactly or approximately, in any other) that were filtered against a repeat database (http://int-citrusgenomics.org/usa/ucr/Files.php). For 8,786 unigenes, no unique oligos are found, either because the C38 assembly occasionally places alternate alleles in different unigenes, or because very similar paralogs occur in the citrus genome. In these cases OligoSpawn software selected 36-bp oligonucleotides which occur in more than one putative unigene, resulting in a total of 31,230 unigenes covered by overgo probes. For BAC library screening we used 89 overgo probes associated with unigenes that putatively code for structural enzymes of the selected pathways (flavonol, anthocyanin, carotenoid, chlorophyll, cellulose, starch, ascorbic acid, aromatic amino acid and lignin biosynthesis; sucrose catabolism; glycolysis; oxidative/nonoxidative pentose phosphate pathway; fatty acid biosynthesis and oxidation; Krebs cycle). In this paper, we describe the methods and we report on experimental results for the overgo probes designed.

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Poster Abstract – D.64

METABOLIC ENGINEERING OF TOMATO FRUIT CAROTENOIDS INDUCES GLOBAL CHANGES IN FRUIT GENE EXPRESSION, VOLATILE AND PRIMARY METABOLITE PROFILES

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carotenoid, tomato, ripening, metabolomic, microarray

We performed a global profiling of tomato fruits overexpressing lycopene beta-cyclase (*LCY-b*), beta-carotene hydroxylase (*CHY*) or both transgenes, under the control of the chromoplast-associated *PDS* promoter. All fruits show large alterations in carotenoid composition and in . endogenous carotenoid gene expression. Variations in carotenoid composition are associated with large modifications in transcriptome profiles, affecting regulatory genes (myb and zinc-finger transcription factors) and genes involved in primary and secondary metabolism and hormone biosynthesisDramatic alterations were also observed in volatile and primary metabolite profiles. All fruits exhibited severe ripening phenotypes, with alterations in ethylene production and fruit firmness. Overall, these data indicate that carotenoid metabolism plays a hitherto unsuspected role in the control of tomato fruit metabolism and ripening.

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Poster Abstract – D.65

CHARACTERIZATION OF A BOWMAN-BIRK INHIBITOR FROM LENTIL: EXPRESSION AND ANTITUMORAL PROPERTIES

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Bowman-Birk, expression, chymotrypsin inhibitor, anti-tumoral properties, lentil

Proteinase inhibitors are widely diffused in plants. In legumes, mainly two types of these inhibitors have been identified: the larger Kunitz inhibitor (16-21 kDa) generally with two disulfide bonds and one reactive site for trypsin or chymotrypsin, and the smaller (6-9 kDa), double-headed Bowman-Birk one (BBI), generally with seven disulfide bridges, a high cysteine content and two reactive sites, one for trypsin and the other for trypsin or chymotrypsin. These two reactive sites are separately distributed in two homologous active loops on the same polypeptide chain and can interact simultaneously and independently with two target proteases.

In plants, the trypsin inhibitor site has the ability to inhibit animal digestive enzymes, thus representing an ideal candidate to protect plants against insect predation through genetic engineering, but may also be involved in the regulation of endogenous plant proteases.

On the other hand, the chymotrypsin inhibitor site seems to be involved in the prevention or suppression of carcinogen-induced transformation *in vitro* and of carcinogenesis in animal model systems. Moreover, BBIs have also displayed anti-inflammatory activity and have been tested in the treatment of experimental autoimmune encephalomyelitis, an animal model disease for human multiple sclerosis.

Two BBI gene classes have been reported in lentil, one coding a trypsin/trypsin inhibitor, the other encoding a trypsin/chymotrypsin inhibitor, even though the sequence of the latter was not complete at the 3' end. We isolated a complete cDNA sequence coding for lentil trypsin/chymotrypsin BBI. The inhibitor was expressed in the methylotrophic yeast *Pichia pastoris*. After purification, recombinant molecules were analysed by MALDI-TOF mass spectrometry, and the inhibitory activity evaluated, by means of enzymatic assays using specific substrates for trypsin or chymotrypsin. The expressed lentil BBI showed an inhibitory activity similar to BBIs from other plants. The ability of lentil BBI to modulate the viability of human colorectal adenocarcinoma HT29 cells *in vitro* was also assessed.

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Poster Abstract – D.66

ISOLATION OF A GERMACRENE-A SYNTHASE SEQUENCE FROM GLOBE ARTICHOKE

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Cynara cardunculus L., *sesquiterpene lactones, germacrene-A synthase*

The globe artichoke (*Cynara cardunculus* var. *scolymus* L.), a traditional component of the Mediterranean diet, contains bitter principles like sesquiterpene lactones (SLs); among them the most important are cynaropicrin and grosheimin, which explicate immuno-modulatory effects on the human immunitary system and prevent the invasion, migration and metastasis of leukocyte cancer cells.

Cynaropicrin and grosheimin belong to germacranolides, a complex family of molecules widespread in the *Asteraceae (Compositae)*, which are thought to have originated from a common germacrane precursor: (+)-germacrene-A. To date, information concerning the gene responsible for the germacrene-A synthesis are available from chicory and *Artemisia annua*, however no information are available on genes involved in the additional steps (oxidation and cyclisation) required to convert germacrene-A to specific SLs (germacranolides, guaianolides and eudesmanolides).

We report on the isolation of germacrene-A synthase sequences in globe artichoke. Presumably, plant belonging to the *Asteraceae* family share common ancestor enzymes for the early steps of SL biosynthesis. Indeed, a comparative sequence analysis among the *Asteraceae* species showed similarity values up to 100% between available sequences from globe artichoke and the ones derived from yellow starthistle (*Centaurea solstitialis*) and safflower (*Carthamus tinctorius*). We retrieved germacreneA-Expressed-Sequence Tags from the EST database (http://compgenomics.ucdavis.edu) of starthistle and safflower (23k and 19k UNIGENES, respectively) by means of tBLASTn algorithm. Degenerate primers were designed on 6 conserved nucleotidic regions and were applied in PCR reactions using cDNA or gDNA of globe artichoke, as templates.

Three fragments of expected lenghts were obtained by PCR amplification of foliar cDNA. They were cloned, sequenced and, when analysed by means of BlastX algorithm (non redundant protein database, Viridiplantae), they revealed very high identity values (from 84% to 93%) to the GAS gene described in chicory. Due to the presence of introns larger fragments were obtained using gDNA as a template.

The full length isolation of the globe artichoke GAS gene is currently in progress by applying 5'- and 3'-RACE; its enzymatic activity, as well as its expression in different plant tissues will be investigated.

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Poster Abstract – D.67

AN OPTIMIZED, CHEMICALLY REGULATED GENE EXPRESSION SYSTEM FOR *CHLAMYDOMONAS*

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Cytochrome c_6, luciferase, nickel, copper, chelating agents

Chlamydomonas reinhardtii is a model system for algal biology and is used for biotechnological applications, such as molecular farming or biological hydrogen production. The *Chlamydomonas* metal-responsive *CYC6* promoter is repressed by copper and induced by nickel ions. However, induction by nickel is weak in some strains, poorly reversible by chelating agents like EDTA, and causes, at high concentrations, toxicity side effects on *Chlamydomonas* growth. Removal of these bottlenecks may encourage the wide use of this promoter as a chemically regulated system for the expression of heterologous genes. Using a codon-optimized *Renilla* luciferase as a reporter gene, we explored several strategies to improve the strength and reversibility of *CYC6* promoter induction. Use of the first intron of the *RBCS2* gene or of a modified TAP medium increases the strength of *CYC6* induction up to 20-fold. In the modified medium, induction is also obtained after addition of specific copper chelators, like TETA. At low concentrations (10 µM) TETA is a more efficient inducer than Ni, which becomes a very efficient inducer at higher concentrations (50 µM). Neither TETA nor Ni show toxicity effects at the concentrations used. Unlike induction by Ni, induction by TETA is completely reversible by micromolar copper concentrations, thus resulting in a transient "wave" in luciferase activity, which can be repeated in subsequent growth cycles.

In the currently used system, downregulation of Photosystem II activity, leading to hydrogen production, is triggered by cycling *Chlamydomonas* cultures between sulphur-replete and sulphur-depleted medium. The method presents evident challenges, such as the difficulty of centrifuging the huge volumes of algal cultures needed for making hydrogen production economically interesting. The use of the TETA/Cu reversible system described here could be used to trigger several subsequent cycles of gene expression/silencing in a cheap, energy-efficient way.

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Poster Abstract – D.68

CHARACTERIZATION OF OIL PALM IN VITRO REGENERATION SYSTEM: MICRODENSITOMETRIC ANALYSIS IN REPRODUCTIVE AND DEVELOPMENTAL PROCESSES

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oil palm, somaclonal variation, meiosis, microdensitometric analyses

Oil palm (Elaeis guineensis J.) represents the most important plant cultivation in the majority of subtropical regions of the Asiatic continent, as well as the derived palm oil is mainly used in diet and in biofuel production. For the economic importance of this cultivation, many Asiatic Companies are interested in *in vitro* culture of oil palm, particularly in the propagation of the most productive F1 genotypes (Dura x Pisifera); however *in vitro* cultivation induces a large number of unproductive somaclonal variants and the ones affected in floral and fruit formation (mantled phenotype) can be identified and eliminated only at sexual maturity after 5 years of field growing.

Since 1996, our group has been collaborating with Malaysian Palm Oil Board (MPOB) to characterise the *in vitro* regeneration system of oil palm. Several approaches were performed to analyze cytogenetic and molecular phenomena, occurring during the proliferation and the regeneration processes, confirming the presence of a gametic-like reprogramming in the acquisition of embryogenic capacity (Geri et al. 1999; Giorgetti et al. 2007a). In order to analyze the expression of some oil palm floral genes, experiments of *in situ* hybridization were performed in floral-like structures and in *in vivo* inflorescences of normal and mantled plants (Giorgetti et al. 2007b). Moreover, a study of meiosis by FISH analyses showed the presence of DNA extrusion of specific sequences (repetitive sequences) and validated the idea of a DNA modulation content during the development *in vivo* as *in vitro*. To confirm this phenomenon, an extensive microdensitometric analysis was performed during the progression of meiosis as well as during the establishment of new embryogenic culture, on new regenerated plants at different times, and finally on seedling obtained from adult regenerated normal and abnormal mantled plants.

A variable loss of DNA content/nucleus in regenerated plants was demonstrated, in some case very severe but no variation was present in seedlings (open pollinated derived) from adult regenerated mother plants. The clear cut information, coming from these data, is that the regeneration process is imputable to induce a DNA modulation/loss per cell in the regenerated plants but progressively DNA sequences are regained before the meiotic process completion. This information, once definitively confirmed, point out directly to mechanisms of somaclonal variation.

Geri C *et al.*,1999, Genome, 42 (6):1134-1143.
Giorgetti L *et al.*, 2007a, Caryologia 60(3): 279-289.

Giorgetti L *et al.*, 2007b, Proceedings of the PIPOC 2007 International Palm Oil Congress (Agricolture, Biotechnology & Substainability) 26-30 August , Kuala Lumpur Convention Center, Malaysia, Vol. I pag 277-298.

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Poster Abstract – D.69

INTERACTIONS BETWEEN YEAST MITOCHONDRIAL AND NUCLEAR GENOMES: THE LYCORINE RESISTANCE IN THE RETROGRADE REGULATION

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Saccharomyces cerevisiae, lycorine resistance, retrograde regulation, RTG genes, mitochondrial DNA polymerase γ

Mitochondrial (mt) genomes of the yeast *Saccharomyces cerevisiae* can exist in different states: rho^+ (wild-type), mit^- (mt point mutations), rho^- (partial deletions of mtDNA), and rho^0 (entirely lacking mtDNA). Cells are able to monitor and to respond to the different functional states of their organelles. Yeast cells respond to mitochondrial dysfunction by altering the expression of a subset of nuclear genes. This response, called retrograde regulation, functions to better adapt cells to mitochondrial defects (Butow and Avadhani, 2004). In derepressed, respiratory-deficient cells, the expression of genes involved in anaplerotic pathways, transport of small molecules, peroxisomal activities, and stress response are up-regulated. Expression of these genes is activated in cells lacking mitochondrial function by involvement of *RTG1*, *RTG2*, and *RTG3* genes whose protein products bind to "R-boxes" in the promoter region. Rtg2p plays a pivotal role in the retrograde pathway because it is both a sensor of the functional state of mitochondria and is required for the activation of RTG-dependent gene expression by promoting the cytoplasmic-to-nuclear translocation of Rtg1p and Rtg3p.

Previously we have demonstrated that the lycorine, an alkaloid of the family of Amaryllidaceae, is able to differentiate between cells devoid of mtDNA (rho^0) and cells with mtDNA, either rho^+ or mit^- or rho^-. Wild-type rho^+ cells, rho^- cells, and mit^- cells are all sensitive to lycorine; rho^0 cells, however, are resistant to high concentrations of the drug. Since rho^0 strains, lacking the entire mitochondrial genome, are in the higher dysfunctional mitochondrial status, we have analysed a retrograde regulation process by lycorine resistance in rho^0 cells. To this aim, we have analyzed the growth of rho^0 *Δrtg* strains, deleted in *RTG* genes, in presence of lycorine. We found that rho^0 *Δrtg* mutants, lacking of the RTG retrograde regular products, were sensitive to lycorine as well as rho^+ yeast strains. Our data on lycorine point to a signalling of mitochondria to nucleus. Since the growth of rho^+ cells is inhibited in presence of lycorine both in glucose and glycerol medium, it could be hypothesized that in rho^0 cells the dysfunctional mitochondrial status stimulates overexpression of nuclear genes very likely involved in both nuclear and mtDNA replication.

A principal candidate among the latter nuclear genes is the mtDNA poly γ gene. It has been hypothesized an additional role of mtDNA poli γ protein besides mtDNA replication since it is stable in the absence of the entire mtDNA in rho^0 cells. In our case, the presence or the absence of mtDNA influences the expression of this gene(s) and it is mediated by the retrograde response, in

fact, we have shown that the lycorine-resistant phenotype is expressed in rho^0 strains only in the presence of RTG nuclear background (Del Giudice et al., 2005).

- Butow RA, Avadhani NG (2004). Mitochondrial Signaling: The Retrograde Response. Mol. Cell. 14: 1-15
- Del Giudice L. et al. (2005). Gene 354: 9-14

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Poster Abstract – D.70

GENETIC TRANSFORMATION OF S*ACCHAROMYCES CEREVISIAE* WITH PLANT GENES: PRODUCTION OF CONJUGATES WITH HYDROXYCINNAMATES

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phenylpropanoid pathway, 4CL, HCT, Saccharomyces cerevisiae

The phenylpropanoid phenolic acids are important components of the antioxidant activity and therapeutic properties displayed by certain plant extracts. The phenylpropanoid pathway catalyzes the conversion of phenylalanine to secondary metabolites, such as hydroxycinnamic acid. Esters formed by the reaction between hydroxycinnamate and particular organic acids (e.g., shikimic and quinic acid) represent a major family of plant phenol acids, with chlorogenic acid as the most naturally abundant molecule.

In plants, the synthesis of phenolic acids can be mediated by the enzymes 4CL (4-coumarate: CoA-ligase) and HCT (hydroxycinnamoyl-CoA:shikimate/quinate hydroxycinnamoyltransferase). The former catalyses the conversion of *p*-coumarate and some related substrates (caffeate and ferulate) to their respective CoA esters; while the latter can use either *p*-coumaroyl-CoA or caffeoyl-CoA as its substrate, and in the presence of either quinate or shikimate, acts to promote the synthesis of either caffeoylquinate (chlorogenic acid), coumaroylquinate, caffeoylshikimate or coumaroylshikimate.

We describe here a yeast-based (*Saccharomyces cerevisiae*) production system of conjugated hydroxycinnamate, founded on the heterologous expression of tobacco 4CL and globe artichoke HCT, we previously isolated. Unlike wild type yeast strains, the transformed yeast cultures metabolized both caffeic and *p*-coumaric acid. Incubation with *p*-coumaric acid produced a detectable level of coumaroylshikimate, the expected reaction end product in the presence of both 4CL and HCT. However, the predominant compounds formed after a 72h incubation with *p*-coumaric and caffeic acid were two highly hydrophobic molecules of molecular mass 298 and 314. We assume that these represent the condensation products between *p*-coumaroyl-CoA and caffeoyl-CoA together with one molecule of 3-hydroxyanthranilic acid. These molecules resemble the avenanthramides, which have anti-carcinogenic and anti-inflammatory activity. To assess the activity of globe artichoke HCT, the relevant cDNA was cloned and heterologously expressed in *E. coli*. This assay confirmed that caffeoyl-CoA can act as a donor molecule in the presence of hydroxyanthranilate.

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Poster Abstract – D.71

OLIVE FRUIT TRANSCRIPTOME ANALYSIS THROUGH 454 PYROSEQUENCING

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454 sequencing, EST, transcriptome, Olea europaea, secoiridoids

The application of a massively parallel pyro-sequencing technology (by 454 Life Sciences Corporation) provides hints to improve the characterization of the olive fruit mesocarp transcriptome, with a focus on terpenoid metabolism, due to the importance of these compounds on the mechanism of pathogen resistance and their effects on human health.

Four different cDNA libraries were prepared applying the SMART technology (Clontech) from fruits of a cultivar characterized by a high oleuropein (main terpene secoiridoid) content and an oleuropein-lacking natural variant, respectively at the beginning and at the end of fruit development.

Five µg of each purified cDNA library were provided for 454 sequencing.

A total of about 260,000 reads were generated for all of the samples with a total output of 58 Mb. Between 50,000 and 77,000 reads were obtained for each sample. The average read lengths were between 217 and 224 bp. Quality filtered sequences from whole shotgun sequencing were *de novo* assembled, obtaining contigs up to 3,920 bp long.

The ParPEST (Parallel Processing of ESTs) pipeline has been tweaked and redesigned in order to manage assembled 454 data. EST assemblies will be annotated and classified according to their biological functions. In addition, we are going to evaluate and compare gene expression patterns as they reflect the composition of the EST libraries prepared from different genotypes and developmental stages. Raw data processing is in progress and results will be adequately reported and organized into a MySQL relational database. Finally, an user-friendly web interface will be created to allow data browsing.

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Poster Abstract – D.72

ANALYSIS OF SWEET ORANGE FLESH PROTEOME AT RIPENING TIME AND COMPARISON WITH TRASCRIPTOMIC DATA

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2D electrophoresis, LC-MSMS, anthocyanins, maturity flesh, Citrus sinensis

In Italy the sweet orange production is characterized by red pigmentation, due to the anthocyanin content. On citrus mature fruits anthocyanins are exclusively expressed in blood oranges and its hybrids.

The characterization of proteins isolated from flesh orange tissue is apparently an essential parameter for understanding orange anthocyanin pigmentation at ripening time.

In this work we present the analysis of a nucellar line of Moro (a blood cultivar) and Cadenera (a common orange) flesh orange using a proteomic approach.

For the fist time we succeeded in extracting the whole citrus flesh proteome following a procedure based on phenol extraction coupled with ammonium acetate precipitation.

Proteome maps obtained by 2D electrophoresis were compared to assess the extent to which protein distribution differs in orange flesh ripe of blood pigmented and common cultivar.

The tryptic digest of the spots differential expressed in the blood (23 spots) and in common oranges (39 spots) were characterized by LC-MSMS and the proteins were identified by searching protein and EST databases.

Proteins involved in stress response (chaperones) and primary metabolism were identified as being over-expressed in Cadenera flesh. Proteins involved in the secondary metabolism, such as anthocyanin's pathway, defence mechanism and primary metabolism were identified over-expressed in Moro cultivar.

Results obtained through proteomic analysis were compared with a previous investigation performed using a transcriptomic approach (Licciardello et al., 2008, Tree Genetics & Genomes 4: 315-331) using a subtracted cDNA library. The comparison with transcriptomic studies evidentiated some discrepancies, confirming the necessity to carry on proteomic analysis and to go deeper in the analysis of protein posttranscriptional and translational modifications. This combined study may reveal the need to associate both methodology approaches to have a general and complete perspective for the specific analysed challenge.

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Poster Abstract – D.73

RISOTILL: A GENETIC RESOURCE FOR THE IMPROVEMENT OF ITALIAN RICE GERMPLASM

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rice, TILLING, functional genomics, reverse genetics, biodiversity

TILLING (Targeting Induced Local Lesion IN Genomes) is a reverse-genetics approach combining chemical mutagenesis with a sensitive DNA screening-technique to identify point mutations in target genes.

In the framework of the VALORYZA project, a TILLING rice population (denominated RISOTILL) derived from Ethyl Methan Sulphonate (EMS) treatment of the variety Volano was created. The Volano italian rice variety was chosen as being representative of the traditional rice quality and of relevance for ongoing breeding programs in Italy. This genetic resource was created from a starting population of 20.000 EMS-mutagenized seeds and is constituted of 1860 M_1 fertile lines. Molecular screening for mutations at 5 agronomically important genes based on the analysis of 8- to 12-fold DNA pools produced from M_2 DNA samples is underway at the PTP Genomics Platform using the validated FLUOTILL protocol.

As a complementary approach, the natural variation of the 5 candidate genes will also be assessed in a panel of 96 rice accessions representing the existing genetic diversity in the Italian germplasm by ECOTILLING technique.

The RISOTILL mutagenized population, although developed for reverse-genetics purposes, is also suitable for forward-genetics analyses and will be tested to identify variants in plant height, resistance to blast, flowering time, amylose content, panicle size, number of spikelets and other yield components.

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Poster Abstract – D.74

PLANT FUNCTIONAL GENOMICS CENTRE: CURRENT STATUS AND FUTURE PLANS

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microarray, transcriptomics, functional genomics

The Plant Functional Genomics Centre, located at the University of Verona, is a structure devoted to the development of cutting edge technologies for the study of functional genomics. After one year and half of activity the Centre is acquiring a leading position in the genome wide transcriptomics analysis. As a member of French-Italian Consortium for the structural and functional characterization of the grape genome, the Centre is carrying on the development of the microarray reference platform for *Vitis vinifera* transcriptome analysis. Moreover, many collaborations have been already established at national and international level for the study of transcriptome in plants, animals and prokaryotic organisms.

The Centre is based on Combimatrix microarray platform which is characterized by an electricity driven *in situ* synthesis technology of 35-40mer oligonucleotides which allows an extreme flexibility in custom chip design. Different versions of the chip are available with 90.000, 12.000 or 4 x 2000 spots for different applications. This innovative technology allows the reuse of the chip up to 4-5 times, with a significant reduction of the analysis costs. We have established a reliable protocol from sample labeling to data analysis both for transcriptome analysis and for the analysis and validation of miRNAs. To asses the reproducibility of the system we performed Pearson correlation plots of data obtained by several hybridizations of the same array with the same sample. A good reproducibility of inter- and intra-chip data (R2=0.97-0.99) have been obtained up to 4-5 hybridizations.

Furthermore, in the next few months new higher density technologies will be acquired and setup such as a NimbleGen platform (2.1 millions of features). This will allow the analysis of even more complex transcriptomes and to setup genome-wide tiling arrays, comparative genome sequencing assays, and the perfoming of exon trapping experiments for deep sequencing of specific genome regions.

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Poster Abstract – D.75

THE ITALIAN CONTRIBUTION TO THE INTERNATIONAL TOMATO GENOME PROJECT

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tomato, chromosome 12 sequencing, BAC

Tomato (*Solanum lycopersicum*) is an economically and nutritionally valuable crop and constitutes a model plant for genetic research of the *Solanaceae* family. Its genome encodes approx. 35.000 genes, which are largely grouped in contiguous euchromatic regions corresponding to approx. 25% of the total 950 Mb genome. An international project is currently under way to sequence the euchromatic DNA of this species on a BAC-by-BAC strategy with Italy undertaking chromosome 12. BACs have been selected *via* genetic (Introgression Line) or cytogenetic (FISH) mapping. Each sequenced anchor BAC serves as a seed from which to radiate out into the minimum tiling path. Identification of the flanking BACs is performed by molecular validation along with analyses based on the use of bioinformatic platforms developed for the project. To date, sequences of 50 BACs representing more than 40% of the euchromatic region of Chromosome 12 are available. A bioinformatic platform has been built in-house to provide a preliminary annotation of the tomato genome. Sequencing and subsequent annotation of tomato genome are providing a reference enabling comparative genomics and systematics amongst higher plants.

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Poster Abstract – D.76

HELIANTHUS BARCODING DATABASE

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sunflower, biodiversity, interspecific hybrids, plastid genome

To make a database of the genus *Helianthus* we used the approach of barcoding which is becoming an increasingly accepted method to distinguish different species. The genus *Helianthus* comprises more than 50 sunflower species, some of them have become important as models for the study of the genetics of adaptation and speciation. Moreover wild species are interesting sources of many useful characters (drought and salinity resistance, source of CMS, disease resistance) for cultivated species. In this study we characterized at the molecular level using two DNA sequences, the nuclear internal transcribed spacer region (*ITS*) and the plastid *trnH-psbA* intergenic spacer, *H. annuus, H. argophyllus, H. tuberosus* and three *H. debilis* ssp. The nuclear and plastid regions were PCR amplified with specific primers and sequenced with an ABI Prism 3730 Automated DNA sequencer. Intraspecific and interspecific sequence variation was evaluated to assess the technique resolution. After sequence editing with specific software (Phred, Phrap and Consed) we was able to distinguish unambiguously each species looking for SNP, InDel and SSR. According to our results both *ITS* and *trnH-psbA* could be the sequences of choice to develop a database of reference sequences to serve as a *Helianthus* library in providing a practical, standardized, species-level identification tool that can be used for biodiversity assessment, ecological studies and analysis of interspecific hybridization.

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Poster Abstract – D.77

SOOLGLE: A WEB SEARCH ENGINE FOR COMPARATIVE GENOMICS IN *SOLANACEAE*

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Solanaceae, comparative genomics, EST survey, transcriptome

Tomato (*Solanum lycopersicon*) and potato (*S. tuberosum*) genomes are currently being sequenced.

Much effort has been invested to sequence ESTs from these solanaceaus species and to identify unigenes. As a consequence, large collections of ESTs are world-wide available because of the wide interest for berry production (tomato) and for tuber (potato) with regards to cultivation for food consumption.

The EST survey of tomato as well as of potato transcriptomes, combined with the knowledge gained from the Arabidopsis genome sequence, can be used to perform large scale analysis and facilitate comparative functional genomics.

In order to achieve this task, we applied an Arabidopsis-based gene and gene family annotation of both the solanaceous transcriptomes and set up a web based search engine to provide the information to the whole interested community.

The results of the analysis can be explored via the *Soolgle* web search engine available at http://biosrv.cab.unina.it/soolgle.

Soolgle provides a quick route to decipher the function of tomato and potato protein transcripts and to identify ortholog sequences among Solanaceae and Brassicaceae plant species. It integrates information from several other databases including TAIR, TIGR, MATDB, NCBI RefSeq. In addition, cross-references to the *in-house* developed databases TomatEST, PotatEST and the *S. lycopersicum* Genome Browser are included.

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Poster Abstract – D.78

TOWARD OLIVE GENOME SEQUENCING: FIRST INSIGHTS INTO THE GENOME ORGANIZATION

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olive, genome structure, sequencing

One of the fastest ways to obtain insights into the structure, organization and sequence composition of a eukaryotic genome is to sequence a library of randomly sheared genomic DNA. In order to characterize the olive genome, *O. europea* (1C = 1400-1500 Mbp), two libraries were constructed from the cultivar Leccino, one genomic and the other hypomethylated. Genomic fragments in the size range of 3-5 Kb were produced by fragmentation of genomic DNA extracted from nuclei of young leaves, and were then ligated into a plasmid. The ligation mix was first used to transform *E. coli* strain DH10β (mrcA, mrcB, mrcC, mrr) and produce a random genomic library, and inserts from about 3500 clones were selected for sequencing from both ends. The same ligation mix was then used to transform E. coli strain DH5α (MrcA, MrcB, MrcC, Mrr). Since restriction systems for methylated DNA are intact in this strain, so that DNA fragments containing methylated inserts are less likely to survive the cloning process. About 1000 clones from this second library (methyl-filtered library) were sequenced from both ends.

About 5,2 Mbp of sequence was obtained from the random genomic libraries, corresponding to 0,35% of the olive genome. A bioinformatic pipeline has been used to analyse the sequencing data in order to have a first insight into the olive genome structure useful to define strategies for a possible *O. europea* genome sequencing project. Surprisingly, the olive genome differs with respect to the large genome characterised until now for the relative low percentage of transposable elements and the very large number of tandem repetitive sequences (satellite/minisatellite DNAs) which seems to be the major responsible of the olive large genome size.