SETTING-UP OF LASER MICRODISSECTION FOR CAPTURING PLANT MALE MEIOCYTES

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LCM, meiosis

So far, plant meiotic genes have been isolated through comparative analysis with model organisms like yeast and drosophilas or, alternatively, by forward genetics approach in Arabidopsis thaliana mutants. We are setting up a methodology which combines the single cell technology based on Laser Capture Microdissection (LCM) and the microarrays for a large-scale analysis of gene expression to elucidate the meiotic process.

Arabidopsis thaliana ecotype Col-0 was grown at controlled conditions and floral buds were processed by LCM to collect meiotic and somatic cells. In order to preserve the morphology of floral structure the tissues were fixed in Farmer’s solution and infiltrated in 30% sucrose to be criosectioned. Meiotic stages were recognized on few sections of each floral bud stained with 4-6-diamidino-2-phenylindole (DAPI) and the cell capture was performed on unstained sections of the corresponding sample. LCM Arcturus Pix Cell II system was set up by evaluating parameters of “capture efficiency” to isolate somatic cells from sepals and meiocytes from anthers and the number of captured cells to be used for RNA extraction. RNA yield and quality was evaluated according to nanodrop ND-1000 and Agilent Technologies, respectively, and RNA specificity was verified through RT-PCR. LCM system parameters are the same for both somatic and meiotic cells. In particular, power range was 90-100 mW, duration 1.0-1.5 ms and spotsize 7.5 µm. The number of somatic cells is about ten thousand to recover 10-20 ng/µl RNA while the meiocyte number is about 4000 to obtain 7-15 ng/µl RNA. The ratio 28S/18S indicating RNA quality is fair. To verify the absence of somatic cell contamination of meiocyte population and the presence of meiocytes at early meiotic stages, when polyA+RNA is abundant, RT-PCR amplification analysis was carried out with a tapetal specific marker (ATA7) and with two meiotic genes expressed at prophase I stage (SWI1 and DMC1). The following step is to obtain RNA amplification to allow the microarray analysis.
EST DATABASE: MINING ON EXPRESSION PATTERNS

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Solanaceae genomics, tomato genome sequencing project, bioinformatics platform, multi-level computational environment

The success of the bioinformatics approaches is directly dependent on the efficiency of data integration, which in turn is determined by the diversity of data sources, the quality of their annotation and the level of details of the information produced.

Here we present results from Plant EST (Expressed Sequence Tag) database analysis to support expression patterns detection from organism specific libraries.

EST collections are certainly no substitute for a whole genome scaffold and show high levels of sequence redundancy and low quality sequence attributes. However, they currently represents the core foundation for understanding genome functionality and the most attractive route for broad sampling of transcriptome from specific libraries.

Here we discuss our strategy to enhance data quality and increase data information content. Moreover, we describe our effort to exploit data classification and the analysis of co-expressed genes. Preliminary results and possible strategies to select genes of specific interest are discussed.
ISOL@: AN ITALIAN SOLANACEAE GENOMICS RESOURCE


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Solanaceae genomics, tomato genome sequencing project, bioinformatics platform, multi-level computational environment

The long term goal of the International Solanaceae Genome Project (SOL) is to exploit the information generated from the Tomato Genome Sequencing Project for the analysis of the genome organization, the functionality and the evolution of the entire Solanaceae family.

To address key questions risen by the vision of the SOL project, large amounts of data from different ‘omics’ approaches are being generated. The raw data are hardly useful as they are and need to be converted into biological meaningful information. Therefore, bioinformatics approaches become preeminent, though their results may be far from being exhaustive and complete.

The success of the bioinformatics approaches is directly dependent on the efficiency of integration, which in turn will be determined by the diversity of data sources, the quality of their annotation and the level of details of the information produced.

Here we present ISOL@, an Italian SOLAnaceae genomics resource available at http://biosrv.cab.unina.it/isola

ISOL@ is a multi-level computational environment which has been conceived in order to effectively integrate genome information with expression, biochemical and metabolic data.

ISOL@ is currently organized into two main levels: the genome and the expression levels. The cornerstone of the genome level is represented by the tomato genome draft sequences. The founding elements of the expression level are the Solanaceae EST collections and the oligonucleotide probe-sets which have been generated for the production of the tomato expression microarrays.

‘Basic’ tools are designed and included into the multi-level environment for enhancing data quality and increasing data information content. ‘Subsidiary’ tools lay over the existing multi-level environment exploiting the sinergy between the levels.

A nonstop crosstalk between the genome and the expression levels is based on the data source sharing and on tools which accomplish the integration of the information which belongs to the respective under parts.

Each level can be independently accessed through specific Web interfaces. The tomato Genome Browser Gateway (genome level) or the EST Database Gateway (expression level) are, to date, the entry points which allow user-driven data investigation.

ISOL@ has been designed so that the existing multi-level environment could be extended to the proteome and metabolome levels through pre-defined entry points.
CHARACTERIZATION AND EVOLUTION OF THE CELL CYCLE-ASSOCIATED MOB DOMAIN-CONTAINING PROTEINS IN EUKARYOTES


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* Mob genes, phylogenesis, cytokinesis, apoptosis, morphogenesis

The MOB family includes a small family of cell cycle-associated proteins, approximately 210 to 240 amino acid residues in length, highly conserved throughout eukaryotes. Its founding members are known to be implicated in mitotic exit and co-ordination of cell cycle progression with cell polarity and morphogenesis. We report the characterization and evolution of the MOB domain-containing proteins as inferred from the 43 eukaryotic genomes so far sequenced.

Our results show that genes for Mob-like proteins are present in at least 41 of these genomes, confirming the universal distribution of this protein family and suggesting its prominent biological function. The phylogenetic analysis reveals five distinct MOB domain classes, showing a progressive expansion of this family from unicellular to multicellular organisms, reaching the highest number in mammals. Plant Mob genes appear to have evolved from a single ancestor, most likely after the loss of one or more genes during the early stage of Viridiplantae evolutionary history.

Three of the Mob classes (i.e. Mob1, Mob2 and Mob3) are widespread among most of the analyzed organisms. The Mob1 class contains two subgroups (A and B): Mob1A includes the ortholog of S. cerevisiae in fungal species and single proteins from H. sapiens and D. melanogaster, whereas the Mob1B group contains one or more proteins from H. sapiens, D. melanogaster, D. rerio, C. elegans and X. laevis. Compared to the other Mob classes, the Mob2 class presents a lower gene identity percentage homogeneity, revealing the possible presence of other subgroups belonging to this class. The Mob3 class is the most divergent one, suggesting a possible different function for the genes belonging to this class. Moreover, phylogenetic analysis shows that the Mob4 genes form a peculiar class of the invertebrata taxa, that underwent an expansion in vertebrata giving origin to Mob4A and Mob4B subclasses.
The possible biological and molecular function of Mob proteins and their role in conserved signaling pathways related to cell proliferation, cell death and cell polarity in eukaryotes are also presented and critically discussed.
LARGE-SCALE GENE ONTOLOGY ANALYSIS OF PLANT GENOME AND TRANSCRIPTOME SEQUENCES RETRIEVED BY AFLP TECHNOLOGY

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GO, annotation, genomic AFLP, cDNA-AFLP, ESTs

After 10-year-use of AFLP 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 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.

We retrieved from both NCBI databases and private repertories a total of 7,806 cDNA-AFLP sequences related to roots, leaves, stems, flowers, fruits and seeds, along with the 285 publicly available genomic AFLP sequences. All these entries belong to 22 different species distributed among seven botanic families: Solanaceae, Fabaceae, Poaceae, Salicaceae, Rosaceae, Brassicaceae and Vitaceae. Redundant sequences were preliminarily clustered to select singlets and assemble contigs. BlastX analysis against non-redundant protein databases, GO terms mapping and annotation analysis were then performed using Blast2GO, a research tool designed with the main purpose of enabling GO based data mining on sequence sets for which no GO annotation is yet available. Descriptive statistics on the type, size and nature of chromosome regions and gene sequences mainly investigated using the AFLP technology were calculated. The gene sequences associated to mRNA transcripts and proteins were classified according to the GO vocabularies. In addition to cellular component, biological process and molecular function, other hierarchically structured GO terms were adopted to query sequences and to assign genes and gene products at different levels, depending on the depth of knowledge. A classification of all cDNA-AFLP records for the main GO vocabularies was also performed by splitting the sequence dataset in monocots and dicots and by comparing the two subgroups with all annotated ESTs of Arabidopsis and rice.

The examination and annotation of EST clones enabled basic inferences to be made on the potentials and drawbacks of AFLP technology for mRNA profiling and differential display gene cloning. Although the different number of sequences retrieved in gene banks for plant organisms and organs might have biased some of the descriptive statistics, the whole set of data emerged from gene ontologies is consistent with the existence of AFLP features exploitable across plant transcriptomes (e.g., ESTs associated to kinase activity can be assayed with very similar rates (11%) in each of the families analyzed in this study) and supports the reliability of expression patterns detection using very small amounts of messengers (i.e., DD-cDNA-AFLP applied to tissues where it is hard to isolate stage-specific mRNAs, such as flowers, fruits and seeds). On the whole, experimental steps and statistical parameters adopted for the in silico AFLP technology-derived sequence analysis proved to be critical for obtaining robust ontology data. Annotation results for the
whole sequence dataset and also for botanic families, single species and plant organs are presented and the main features of genes and gene products detectable in plants by genomic AFLP fingerprinting and cDNA-AFLP profiling discussed. To the best of our knowledge this is the first large-scale survey of amplified fragment length polymorphism-derived sequences belonging to plant angiosperms.
PLEIOTROPIC EFFECTS OF A 69 BP DUPLICATION IN THE ORF OF ADH1 GENE OF SACCHAROMYCES CERAVISIAE


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alcohol dehydrogenase, DNA duplication, yeast, SWI1, respiratory activity

ADH1 gene codes for a cytoplasmic alcohol dehydrogenase fermentation isozyme. It is known that S. cerevisiae strains, resistant to allylic alcohol, can carry mutations in the ADH1 gene. Spontaneous mutants, resistant to allylic alcohol, have been isolated from the wine yeast, S. cerevisiae 1014. Cells of the selected mutants, when grown on medium containing glucose as energy source (YPD), develop colonies of different size, “large” and “small”; cells from large colonies produce large and small colonies, while cells from small colonies produce only small colonies. The growth rate of the resistant mutants is reduced, mainly in cells from small colonies; when grown on a medium with an energy source not fermentable (YPG), only small colony are produced. Cells from small colonies as well as cells of petite mutant resistant to allylic alcohol do not grow at 37°C. Cells from hybrids, carried out between the parental strain and the resistant mutants, when grown on glucose supplied medium originate only large colonies, suggesting that the mutation is recessive. Analysis of tetrads, derived from the hybrids, has shown that the phenotypes, large colony and small colony, segregate in the 2:2 ratio, suggesting that allylic alcohol resistance, in the analysed strains, is due to a single gene mutations.

In one of the mutants the ADH2 sequence analysis has shown that the mutation is a duplication of 69 bp localized in the orf of the gene, which is present in both the DNA from large and small colonies. During the growth a significant number of cells of the mutant loose the 69 bp duplication, both at 28°C or 37°C, with recovery of the wild type phenotype (resistance loss and production of colonies of the same size). Gene expression analysis, carried out in cells grown in both media (YPD and YPG), has shown that the mutated ADH1 gene is expressed in the cells of parental strain, of the hybrid and in the cell from large and small colonies derived from the tetrads. The mutated Adh1 protein was also found functionally active under the same cell growth conditions. No ADH1 gene expression neither active Adh1 protein was found in cells from a petite mutant resistant to allylic alcohol. ADH2 gene was not expressed in all mutant cells, this could depend on the lack of the expression of the transcription factor SWI1 involved in the ADH2 gene regulation. When the mutation reverts the expression of ADH2 and SWI1 is again recovered. Mitochondrial gene expression and energy activity were also investigated in wild type and mutants strains.
SEARCH FOR DIAGNOSTIC TOOLS IN *Fusarium oxysporum* f. sp. *melonis* RACE IDENTIFICATION


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plant disease, soilborne pathogen, molecular characterization, melon

*Fusarium oxysporum* f. sp. *melonis* is the most destructive fungal pathogen of melon in all growing areas. A collection of a total of 39 isolates of *F. oxysporum* f. sp. *melonis*, representative of all four known races (0, 1, 2, and 1.2) was analyzed by several molecular techniques in attempt to characterize the *forma specialis* and the races. The principal target was to discriminate race 1,2, the most virulent and widespread race in Europe and Italy. Microsatellites (SSR and ISSR), and calmodulin partial sequence analysis were unable to discriminate. In turn, minisatellites (M13 and T3B), translation elongation factor alpha partial sequence analysis, and RAPDs allowed to clearly differentiate race 2 isolates from all other races, confirming its polyphyletic origin. Race 1,2 isolates clustered together, with few exceptions, in RAPD analysis using 23 primers out of 89 tested. A unique band was identified which was present only in race 1,2 isolates. The corresponding sequence will be used for specific primers design.
EXPLORING THE MOLECULAR BASIS OF INSECTICIDE RESISTANCE IN THE CODLING MOTH CYDIA POMONELLA (L.)

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Cydia pomonella, insecticide resistance

The codling moth (Cp), Cydia pomonella (L.) is a major pest in most pome fruit orchards worldwide. Recently, under the selective pressure caused by an increasing number of chemical treatments the Cp has progressively developed a reduced insecticide sensitivity primarily to organophosphates (acetylcholinesterase inhibitors), and to insect growth regulators (chitin synthesis inhibitors). The adoption of novel pest management strategies directed to circumvent and/or delay resistance outbreaks and to control the insensitive pests require the understanding, at molecular level, of the resistance mechanisms, mainly the insecticide target insensitivity and the enhanced detoxifying metabolic pathways as well as the setting up of suitable diagnostic tests for their in lab detections. In this vein we applied a direct sequencing strategy to detect mutations in the AChE-1 gene responsible for the target site sensitivity. Moreover biochemical assays for the most important detoxifying systems (i.e. esterases, glutathione-S-transferases and mono-oxygenases) were used to drive the cloning of candidate genes for resistant phenotypes. Finally the gene expression of a cloned glutathione-S-transferase and a mono-oxygenase family member was reported at the different life stages in susceptible and resistant Cp strains.
FUNCTIONAL ANALYSIS OF CELL CYCLE-ASSOCIATED MOB1 GENES USING ARABIDOPSIS THALIANA RNA-INTERFERED MUTANTS


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plant reproduction, Arabidopsis, 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. Mob1 proteins have been demonstrated to be important for both mitosis completion and cell plate formation in yeast. Moreover, the Mob1-related proteins Mob2 physically associates with specific kinases throughout the cell cycle, being required and periodically activated in yeast to promote polarized growth. Although there are data to suggest that Mob1-like proteins act as kinase activating subunits in higher eukaryotes, their function remains to be proved. Present findings imply animal and yeast Mob1-domain containing proteins have similar functions.

Plant genomes such as alfalfa, Arabidopsis, rice and Poa pratensis also contain Mob1-related genes. We have recently cloned two Mob1-like genes from Medicago sativa and demonstrated that their proteins are involved in cell proliferation and are localized in the cell division midplane during cytokinesis (Citterio et al. 2006 Exp. Cell Res. 312: 1050-1064). In addition, Mob1-like genes proved to play a key role during the reproductive pathway in plants: localization of their transcripts and proteins was associated to meiotic division abnormalities and programmed cell death within reproductive organs (Citterio et al. 2005 Plant Mol. Biol. 58: 789-807). In particular, 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 apomictic 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.

The aim of our ongoing experiments is to elucidate the role of Mob1-like genes and to gain further insights on their function in plants. Our hypothesis is that Mob1 proteins alone or with protein kinases are involved in cell cycle control, but they seem also associated to programmed cell death into reproductive organs when combined with apoptotic regulators. Functional analysis of Mob1-like genes of A. thaliana (loci At5g45550 and At4g19050) was attempted by using RNA-interfered Mob1 mutants. Silenced single-insertion homozygous lines are being investigated on the
basis of plant morphological traits and cytohistological observations of female meiosis and gametogenesis, and ovule development. Preliminary data support an altered growth habit and a strongly reduced seed set in AtMob1-interfered plants. In particular, a faster development of plants along with thinner shoots and smaller flowers and siliques were observed. Moreover, most pollen grains were apparently non-viable and ovules were shown to contain coenocytic megaspores and nonpolarized embryo sacs. Results on temporal and spatial gene expression patterns of AtMob1-like genes are also reported and their potential role in plant development and reproduction discussed.
COMBINING EVIDENCES FOR REPEATS AND TRANSPOSABLE ELEMENTS ANNOTATION

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transposable elements, automatic annotation, grape, repetitive DNA

Within the frame of the grape genome WGS-sequencing project (French-Italian VIGNA consortium), we computationally and manually verified, examined, and annotated the repetitive fraction, with special focus on Transposable Elements (TEs).

We developed a pipeline for a genome-wide automated annotation of TEs starting from their two basic features: repetitiveness and coding capacity. The common origin and the high copy number of most TEs in eucaryotic genomes were exploited to infer the ancestral sequence of each element, using the dedicated software ReAS. As the ReAS output, especially used with data produced in early stages of the project, needed extensive further consideration and analysis to be used for our purpose---the results consisted mostly in fragmented and uncharacterized highly repetitive elements of the genome---, we began using it to mask the assembly using RepeatMasker. The subsequent steps in our routine consisted in adding and combining further available information, notably presence/absence of coding regions to classify repetitive sequences in TEs. To perform alignments against a selected TE protein database (consisting, in our case, of a curated database of known plant TE protein sequences) we used the BLASTX member of the BLAST family. This second step turned out to be particularly useful in identifying autonomous elements and produced an initial annotation to be manually refined. As an aid for this last activity we developed a tool (MDOTTER, based on DOTTER) capable to automatically show structural features typical of borders of transposable elements, starting from ReAS annotation only. The information to run MDOTTER consists in relative position (e.g. coordinate on a contig) and ReAS unique name sequences. The idea is simply to build a simplified representation of our target string (e.g. our contig) written in an alphabet A where one letter of A will (uniquely) encode a single ReAS sequence. A contig becomes a sequence of words in A, where each word represents a sequence of overlapping repeating fragments. At this point MDOTTER allows both visual and computational analysis calling standard DOTTER after re-encoding of A into an isomorphic version defined in the amino-acids alphabet. Using an ad-hoc cost matrix search, visualization and analysis of a pattern of repeated sequences within contigs become easily performed and computationally feasible.

Apart from autonomous TEs, that are relatively easy to find, non-characterized repetitive sequences and non-autonomous elements were identified based on masking data and MDOTTER evidence only. The resulting annotation was stored as GFF (General Feature Format) and can be visualized on gene browsers like GBrowse and Apollo.
The semi-automatic pipeline was compared with a fully manual annotation and showed good performances. Among the weak points the most significant turned out to be ends determination of low copy-number elements. However, an automated and customizable method to obtain a first estimate of the total amount of TE coverage, number, and diversity is a powerful tool for early stage new genome analysis.
DIFFERENT FAGUS SYLVATICA GENOTYPES UNDER HIGH LEVEL OF CO2: GENE EXPRESSION AND ECOPHYSIOLOGY ANALYSES


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Beech, CO2 response, ecophysiology, microarray

The problems related to global changes mainly caused by human activities, are the origin of much concern for the health of the environment. Oil and carbon combustion, the use of chlorofluorocarbons, and deforestation are some of the principal factors responsible for enhanced CO2 production, as well as for air temperature increases. This scenario could determine global changes affecting precipitation patterns, nitrogen concentration in the atmosphere, UV radiation increase, and temperature range. Forest trees constitute a relevant economic and ecological resource that is under severe treat by environmental changes.

The principal aim is to investigate the response to CO2 from two different F. sylvatica genotypes by gene expression and ecophysiology analyses.

Scions of Fagus sylvatica (Montieri (GR), Italy) and F. sylvatica “purpurea tree” (Grosshansdorf, Germany) were grafted on F. sylvatica rootstocks. Plants were grown under controlled conditions in climate chambers. Air temperature was 25°C during light period and 20°C at night, and humidity 60%. Fluorescent lamps (18 36W) provided a photosynthetic active photon flux density (PPFD) of 250 µmol m⁻² s⁻¹ at plant top level. CO2 concentrations were about 450 ppm (ambient) and 1000 ppm (high) for control and high CO2 chamber, respectively. A PAM fluorescence system (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany) with a 6 mm diameter standard fibre optic was used for the measurements of the in vivo photosynthesis. Light response curves were recorded up to a light intensity of 420 µmol m⁻² s⁻¹. At each step the leaf was illumined for 3 minutes.

Under ambient CO2 concentrations, electron transport rate (ETR) was higher in the Italian compared to the German genotype. After 4 days at high CO2 level, the ETR increased compared to plants growing in the control chamber. Photosynthesis of Italian genotype adapted to 1000 ppm of CO2 decreased immediately after been exposed for 2 hours to 450 ppm CO2. No down-regulation of photosynthesis could be observed in leaves at 1000 ppm CO2 level.

Microarray analyses are in course and preliminary results will be discussed.
THE ENDOPLASMIC RETICULUM CHAPERONE ENDOPLASMIN SUPPORTS THE SYNTHESIS OF CLAVATA3

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Arabidopsis, chaperones, clavata, endoplasmic reticulum

Clavata genes (CLV1,2 and 3) control the size of the shoot apical meristem. CLV3 encodes a small, predicted extracellular protein that is processed into a functional 12 amino acids peptide. This is hypothesized to be a ligand for the CLV1/CLV2 receptor complex on the plasma membrane. The Arabidopsis shepherd (shd) mutant shows phenotypes in shoot apical and floral meristems similar to those of the clavata mutants and is not affected by the overexpression of CLV3, which instead abolishes shoot apical meristem activity in wild type Arabidopsis. The shd defect consists in the greatly reduced synthesis of endoplasmin, because of a T-DNA insertion. Endoplasmin is a molecular chaperone of the heat-shock protein 90 class located in the endoplasmic reticulum. Thus, genetic evidence suggests a role of endoplasmin in the synthesis of CLV3. We previously showed that a much smaller number of newly synthesized plant secretory polypeptides are detected in association with endoplasmin than with the endoplasmic reticulum heat-shock 70 chaperone BiP. Consistently, the former has in vivo chaperone activity lower than the latter when assayed on the model secretory protein alpha amylase. We compared here the ability of endoplasmin and BiP to support CLV3 synthesis in transiently transfected tobacco protoplasts, under normal conditions or endoplasmic reticulum stress induced by tunicamycin. Because anti-CLV3 antibodies are not available, the experiments were performed using a CLV3-GFP fusion and, as a control, a secretory form of GFP. Our results indicate that, contrarily to what determined for alpha amylase, endoplasmin has a stronger effect than BiP on CLV3 synthesis. The results provide biochemical evidence that endoplasmin plays an important role in the synthesis of CLV3, and are consistent with the hypothesis that this chaperone, which is not present in baker’s yeast, has a major role in tissue-specific secretory activities.

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CHARACTERISATION OF AUTONOMOUS HELITRONS IN MONOCOTS

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helitron, DNA transposon, transduplication, maize

Helitrons, a novel class of eukaryotic transposons have recently been computationally identified in plant and animal species. Unlike other transposable elements, they encode a ‘rolling circle’ replication (Rep) and helicase (HEL) protein, carry conservative ends that are not terminal inverted or direct repeats, and do not cause target site duplication.

In maize, most helitrons are non-autonomous and do not encode Rep/HEL. Instead they usually contain multiple genic segments derived from various genomic locations through the process of transduplication. We set out to identify putative autonomous helitron elements in maize. We identified 2 different families. Similarly to Arabidopsis and rice, some maize autonomous helitrons encoded an RPA-like protein besides the characteristic Rep/HEL. Others encoded a protein that carries a Ulp1 protease domain found also in proteins encoded in a class of autonomous MULEs. The presence of latter autonomous helitrons was subsequently confirmed also in rice.

Closer inspection of maize autonomous helitrons revealed that the helitrons containing Ulp1 domain protein carry in addition many transduplicated genic segments. RT-PCR on mRNA from maize and rice seedlings and roots demonstrated that only the Ulp1, but not RPA containing helitrons actively transcribe the Rep/HEL gene. Analysis of expressed sequences indicate that more than one helitron subfamily is expressed. The Ulp1 protease domain gene was not expressed. On the other hand, some of the transduplicated fragments, fused together through ‘exon shuffling’, appeared to be a part of the transcriptome. Examination of DNA methylation status of helitron in maize demonstrated that autonomous helitrons are methylated like some other transposons in plants. Nevertheless, methylation is not caused by siRNA silencing.

The Rep/HEL expression and sequence conservation support the belief that helitrons continue to be transposed. The finding that elements of different families capable of transduplication (MULEs and helitrons) share a common protein domain may be suggestive of a role in the transduplication process. In addition, the discovery of the autonomous helitrons containing transduplicated genic segments could provide a missing link and clues that will shed light on this widespread phenomenon that shapes organisation, structure and function of genes and genomes throughout the evolution.
NUCLEOTIDE DIVERSITY IN GENIC REGIONS OF THE A GENOME OF WILD AND DOMESTICATED TRITICUM SPECIES

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wheat wild relatives, ESTs, SNPs, genetic differentiation

Two different forms of the A genome of Triticum species are known, the A^m genome of diploid T. monococcum, and the A^u genome of diploid T. urartu, that is the A-genome donor of tetraploid wheats. In the present study, the A genome of both diploid and tetraploid Triticum species was analyzed for SNP detection to investigate how evolutionary events have shaped molecular diversity throughout the process of wheat polyploidization and domestication.

Plant material includes wild relatives and cultivated forms belonging to the following species: T. urartu and T. monococcum (diploids), T. turgidum, T. turgidum dicoccoides, T. turgidum durum and T. dicoccon (tetraploids). Seven genic regions on the A genome chromosomes were chosen among those utilized in previous association studies on drought resistance in durum wheat. Amplicons were sequenced and polymorphisms, both SNPs and indels, were scored.

The genetic diversity structure based on the distinct allele frequencies within the entire germplasm was investigated and five clusters including all samples were identified. All wild and cultivated T. turgidum accessions, with the only exception of the subspecies T. turgidum dicoccoides, appear to belong to one single group. Instead, three out of the four accessions of T. turgidum dicoccoides constitute their own separate cluster. The fourth accession is included in another group together with those T. urartu accessions that have similar geographical origin. All other T. urartu individuals are found in still another distinct cluster, possibly because of their different region of origin. All T. monococcum accessions belong to a fifth clearly separate cluster.

The above mentioned structure was tested by AMOVA analysis. Calculation of Fst among pairs of groups confirmed the clear separation between them (Fst > 0.3). The only two groups that appear to be more similar (Fst = 0.03) are the one including only T. turgidum dicoccoides and the one with all the other tetraploid species. We will present and discuss results on gene diversity (Nei), nucleotide diversity (\(\pi\)) and on the extent of linkage disequilibrium in the A genome of the different species.
IDENTIFICATIN OF DREB2 GENE IN WILD ANCESTORS OF COMMON WHEAT AND ITS VARIATION IN AEGILOPS TAUSCHII

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Dre2, Aegilops tauschii, SNPs, drought tolerance

Drought, salt and cold are main abiotic stresses which effect crop production.

Transcriptional genes such as Dreb (Dehydration Responsive Element Binding) gene family have crucial role in promoting the expression of genes associated with these stresses. Expression patterns of these transcription factors of wheat and maize are different from those in rice and Arabidopsis.

Wheat is the most important cereal affected by drought in the most its planting area. Wild relatives are vital sources to improve drought tolerance in wheat.

Common wheat is a polyploidy crop containing A, B and D genomes. Most of the diversity in A (Triticum urartu) and B (Aegilops speltoides) genome ancestors has been transferred to durum and common wheat. Conversely, Aegilops tauschii, the donor of the D genome, has contributed very little, in spite of its significant role in drought tolerance in wheat.

The aim of the present study is the identification and analysis of Dreb2-like genes in common wheat and Aegilops tauschii.

Dreb2-like genes were isolated from the A, B and D wheat donor species. The results confirmed that the gene is composed by four exons, the existence of some similarity between genes in A and D genomes, whereas that in the B genome is different in both length and deduced amino acid sequence. Exon regions were sequenced in 120 genotypes belonging to 40 accessions of Aegilops tauschii, collected from different areas of the species distribution. Different SNPs and insertions were detected in different accessions, promoting non-sense and mis-sense mutations and insertions.
IDENTIFICATION OF GW2 GENE RELATED TO GRAIN WIDTH AND WEIGHT IN A, B AND D GENOME DONORS OF WHEAT

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GW2 gene, grain width and weight, Aegilops tauschi, Triticum urartu, Aegilops speltoides

Many important traits in crops, including yield and stress tolerance, are controlled by QTLs. Grain weight is one of the most important components of grain yield and is controlled by quantitative trait loci (QTLs). Recent studies in rice have succeeded in isolating and characterizing GW2 (Grain Weight gene), a gene that controls grain width and weight.

Here, we report the identification of GW2 homologous gene in A genome (T. urartu), B (Aegilops speltoides) and D genome (T. tauschii). Its sequence shows 70% relative similarity with rice.

GW2 encodes a previously unknown RING-type protein with E3 ubiquitin ligase activity, which is known to function in the degradation by the ubiquitin-proteasome pathway. Loss of GW2 function increased cell numbers, resulting in a larger (wider) spikelet hull, and it accelerated the grain milk filling rate, resulting in enhanced grain width, weight and yield in rice.

Although a thorough understanding of seed development is important for the improvement of grain yield through genetic manipulation, little is known about the genetic mechanisms that determine final seed size and weight in wheat. Also unknown is the variation arose during the formation of polyploids and domestication.

Present findings provide a step in ascertaining gene divergence among wheat ancestors and between them and modern varieties.
MADS-BOX GENES OF MIKC TYPE IN WHEAT (TRITICUM AESTIVUM L.): MOLECULAR AND PHYLOGENETIC ANALYSIS

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wheat, MADS-box genes, phylogenesis, expression analysis, chromosome location

In higher eukaryotes MADS-box genes encode a family of highly conserved transcription factors, which are involved in several developmental processes and in signal transduction. The MADS family includes two main lineages, type I and type II, both represented in plants, animals and fungi. In plants type II genes, or MIKC-type, have extensively been studied, much less is known on type I genes. MIKC genes control flowering induction and morphogenesis of the different flower organs. The interaction of the floral-specific MIKC-type MADS-box genes has been summarised in the ABCDE model of flower development. Moreover, the involvement of MIKC-type genes has been reported in a number of other metabolic processes, suggesting their participation in most aspects of plant development. The aim of the present research is the study of the complexity and diversity of this gene family in wheat. The knowledge of the structural and functional characteristics of these genes will allow the fine-tuning of plant growth and development to specific environments; this will be possible by modulating the extent of the life cycle phases. Moreover, the increase of wheat productivity will be made possible by modifying the spike and flower morphology.

The available sequences of MIKC-type genes of rice (34 sequences) and Arabidopsis (37 sequences) were exploited to BLAST search the public databases of wheat ESTs (Expressed Sequence Tags): TIGR wheat gene index database (TaGI, version 10), HarvEST wheat (version 1.13) and NCBI. BLAST searches identified 29 non-redundant MIKC-type consensus sequences, which were used as templates for 5’ and 3’ RACE (Rapid Amplification of cDNA Ends) extensions. Full-length cDNAs of the 29 putative wheat MIKC-type genes were cloned by RT-PCR of mRNA from various plant tissues using specific primer pairs designed in the 5’ and 3’ untranslated regions. For each of the 29 primer pairs, five independent RT-PCR reactions amplified products with the same electrophoretic mobility, which were cloned and sequenced. All five full-length cDNAs cloned from RT-PCR products exhibited the same sequence for 15 primer pairs, whereas the five clones obtained by each of the remaining 14 primer pairs showed either two or three similar but not identical sequences. Multiple cDNAs cloned from independent amplifications with the same primer pair showed high identity (over 90%) at both nucleotide and amino acid levels, most probably because they derived from transcripts of MADS box genes located in homoeologous chromosomes. Southern analyses showed that in hexaploid wheat there are three homoeologous copies for each of the 29 identified MIKC-type sequence, indicating that the genome of T. aestivum contains at least 87 (29x3) type II MIKC MADS-box genes. This genome organization was further confirmed by aneuploid analysis of six genes assigned to the SEP-subfamily, each showing three copies located in different homoeologous chromosomes. Phylogenetic analysis included the wheat MIKC cDNAs into 11 of the 13 MIKC subclasses identified in plants and corresponding to most genes controlling
the floral homeotic functions. The expression patterns of the cDNAs corresponding to different homoeotic classes was analysed in 18 wheat tissues and floral organs by RT-PCR, real time RT-PCR and northern hybridisation and compared with those of functionally characterized MADS-box genes from *Arabidopsis* and monocot species. Sequence similarity and comparable expression patterns were the parameters used for a preliminary prediction of the potential functions of the genes corresponding to the isolated wheat MADS-box sequences.
A DURUM WHEAT INTERVARIETAL GENETIC AND PHYSICAL MAP BASED ON SSR AND TRAP MARKERS


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durum wheat, markers, molecular maps, physical maps, EST-SSR

The durum wheat is one tetraploide species (genomes AABB) cultivated mainly in the Mediterranean regions, Canada, USA, Argentina and India. In spite of the importance in the worldwide production of wheat, this species has received less attention than common wheat by investigators. Recently genetic maps based on molecular markers (RFLP, AFLP, microsatellites, etc) have been developed and published for the various species of cereals including durum wheat. The availability of genetic maps and phenotypic data of segregant populations allow to localize and map agronomically important genes, and to identify closely associated molecular markers to be used in of marker-assisted selection (MAS) programs and for the positional cloning. Objective of the present work was to develop a durum wheat intervarietal genetic and physical map based on genomic microsatellite markers (gSSR) as anchor chromosome loci, EST-derived microsatellite markers (EST-SSR), and TRAP (Target Region Amplification Polymorphism) markers for the saturation of uncovered chromosome regions. The up to now map comprises 2 morphologic markers (awn colour, bla, and ear glaucousness, W1), two seed storage protein markers (Gli-A2, Gli-B2), 285 SSR and 303 TRAP markers. The JoinMap software grouped the loci in 26 linkage groups, of which 17 assigned to the of the A and B genome chromosomes; the remaining 9 group will be assigned to chromosomes by physical mapping with deletion lines. The number of mapped markers per chromosome ranged from a minimum of 9 markers for the chromosomes 5A and 5B to a maximum of 39 markers for the chromosome 1B, with an average of 25 markers for chromosome. With the integration of the TRAP markers, the basic map accounted for a total length of 1,468.5 cM, with an average density of one marker per 4.8 cM. A higher percentage (57.8%) of the markers has been found localized on the B genome chromosomes, in comparison to 42.2% distributed on the A genome chromosomes. The B genome accounted for 803.3 cM of genetic distance; the A-genome basic maps spanned 665.2 cM. The comparison of the marker order on the chromosomes in the various published maps was made by the common genomic SSR; out of 133 mapped gSSR markers, three markers were found to be localized on different chromosomes.
A DURUM WHEAT LINKAGE MAP: INTEGRATION OF SSR AND DArT MARKERS


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durum wheat, linkage map, SSR, DArT

A genetic linkage map of durum wheat (Triticum turgidum L.) was constructed using segregation data from a population of 176 recombinant inbred lines (RILs) derived from a cross between the cultivars Colosseo (leaf rust resistance donor) and Lloyd (susceptible to leaf rust); the map will be used to identified novel QTLs for leaf rust resistance in durum wheat. A total of 659 loci, including 162 simple sequence repeats (SSRs) and 497 Diversity Arrays Technology (DArTs), were analyzed by means of the package JoinMap V4. SSRs were chosen because they are user-friendly and highly polymorphic, even if they are single-assay technique and the throughput is low. Instead, DArTs offer the possibility to generate hundreds of reliable markers that probe the whole genome of a species with the speed and accuracy of a high-throughput platform. The integrated SSR-DArT linkage map consisted of 633 loci with 162 SSRs and 473 DArTs; 23 DArTs remained unlinked. The markers were grouped into 16 linkage blocks, which spanned a total of 2132 cM, with an average density of one marker per 4.05 cM. SSRs were useful for assigning linkage groups to chromosomes while DArTs allowed linkage group to coalesce and many gaps to be filled in. In this study, we showed that combining the SSR and DArT platforms provides an efficient and rapid method of generating genetic linkage map in durum wheat despite its large genome and its allopolyploid origin.
UTILIZATION OF A DURUM WHEAT GERMPLASM COLLECTION FOR GENE-QTL DISCOVERY USING ASSOCIATION MAPPING


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germplasm collection, association mapping, drought tolerance, durum wheat

A collection of 189 durum wheat accessions chosen to sample the genetic variation present in the major cultivated gene pools (for details, see Maccaferri et al., 2006, Plant Genetic Resources 4: 79-85) has been assembled for genetic association study and allele mining purposes. The collection has been evaluated in 14 field trials carried out under rainfed and irrigated conditions in Italy, Spain, Morocco, Tunisia, Syria and Lebanon for yield, yield stability and morpho-physiological traits related to drought response. The collection has been characterized molecularly with SSR markers covering all the 14 durum linkage groups to evaluate the pattern of long-range linkage disequilibrium (LD) and the presence of population structure. Significant differences among accessions and environments were detected for grain yield, its components and morpho-physiological traits related to drought response. The collection has been characterized molecularly with SSR markers covering all the 14 durum linkage groups to evaluate the pattern of long-range linkage disequilibrium (LD) and the presence of population structure. Significant differences among accessions and environments were detected for grain yield, its components and morpho-physiological traits related to drought response; as an example, grain yield, averaged across environments, ranged from 0.9 to 8.3 t/ha. Marker-phenotype association analysis after accounting for the population structure evidenced a number of chromosome regions significantly associated with each of the measured traits; in several cases the association was consistent across environments. When considering traits characterized by high heritability values, such as plant height, heading date, peduncle length and thousand kernel weight, it was possible to identify a number of SSR markers showing significant associations in more than four (up to ten) environments, with \( R^2 \) values ranging from 5 to 10%. As to yield and its components, the majority of the markers significantly associated with phenotypic values were identified only in two to four environments, with an average \( R^2 \) values lower than 5%. The results obtained in this study have been compared with those obtained in previous linkage-mapping studies conducted in durum and...
bread wheat. This comparative analysis has highlighted the role of a number of chromosome regions that appear as a suitable target of marker-assisted selection for their introgression in elite cultivars lacking the agronomically superior alleles.
MICROSATELLITE MARKERS COMPUTATIONAL ANALYSIS IN WHEAT FROM “TOTIPOTENT” cDNA LIBRARY

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microsatellite, EST - SSRs, cDNA, durum wheat

Simple sequence repeats (SSRs) have become important molecular markers for many applications: genome mapping and characterization, phenotype mapping, marker assisted selection of crop plants and diversity studies.

The exponential growth of wheat published gene sequences along with the high cost of developing plant microsatellite libraries, is facilitating the “in silico” search of microsatellite motifs.

The discovery of SSR markers in ESTs (expressed sequence tags) provided the opportunity to develop microsatellites by the data mining of ESTs databases. This specific approach was first attempted in rice (Miyao et al., 1996) and has subsequently been applied to many other crops.

Nearly 630 SSRs were identified among 9,000 ESTs belonging to a “totipotent” cDNA library (patent n. WO2005003344) of durum wheat (Triticum Turgidum Desf. cv Ofanto).

Among all ESTs from the database, 6.76% contained the SSRs. This is equivalent to 1 SSR per 6.96 kb EST sequence. About 18% of the SSRs were mononucleotide, 26% were dinucleotides, 52% were trinucleotides, and the remaining approximately 4% consisted of tetra-, penta-, and hexanucleotides.

Among the identified SSRs, (CCG/CGG)n was the most frequent (19.87%) followed by (AG/CT)n at 15.58%, (A/T)n at 15.42%, and (AC/GT)n at 7.63%.

PCR primers are being designed and used to search for polymorphic variants on different wheat genotypes.
ADAPTATION TO CLIMATIC CHANGES OF ETHIOPIAN GERMPLASM

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genetic structure, durum wheat, drought

In the last decades the climatic changes are imposing their effects on our life and above all on our alimentary productions. The Mediterranean regions are characterized by the diffused presence of water stress that strongly limits the productions both in quantitative and qualitative terms. The tendency toward a progressive increase of the temperatures and the consequent decreasing of water availability suggests the necessity to found and select genotypes more tolerant to such stresses, which constitutes a priority for the agriculture of the countries in the Mediterranean basin. Ethiopia includes zones that well lent to this type of analysis being an important centre of diversification for the durum wheat and containing a variegated territory both in geologic and environmental terms. In fact, Ethiopia’s territories include zones ranging from very wet to drought. This allows to have a genetic pool extremely variegated to be adapted to very different climatic conditions. In order to determine the adaptation to environmental stress due to climatic changes such as drought, we analyzed a collection of 234 genotypes belonging to nine populations of durum wheat from three Ethiopian regions (Tigray, Gonder and Shewa) that are distinguished for the climatic conditions and for the annual precipitations. This gene pool was analysed by 28 SSRs markers randomly chosen one for each chromosome arm. The results of this study allow us to get information on the genetic structure of the analyzed populations and the degree of polymorphism both inside the populations and among regions. Moreover, the presence and frequency of rare or unique alleles, help us to evidence the existence, in the populations considered, of particular genetic configurations tied to extreme conditions and in the possible location of a more favourable genetic equipment to tolerate such conditions.
HITCHHIKING MAPPING FOR DROUGHT QTL IN ETHIOPIAN POPULATIONS OF DURUM WHEAT

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QTL, durum wheat, hitchhiking, selective sweep, drought

Nowadays that several genetic maps of wheat are available, it is possible to select several loci along a chromosome to discover their distribution of variability and determine the selective sweeps. If a locus is under selection pressure its variability is reduced due to the fixation of the single positive allele. Also the loci nearby the selected ones have a reduction in variability, due to their linkage with it. The neutral variants that are linked to the beneficial mutations are also affected by selective sweep, a phenomenon that has been called hitchhiking. Here are reported the results relative to a durum wheat collection of 234 genotypes from nine populations of three Ethiopian regions: (Tigray, Gonder and Shewa) with contrasting environments, analysed with 25 SSR loci localized on chromosome 4 and used to identify the hitchhiking mapping of this chromosome. The germplasm was chosen since (Triticum turgidum ssp. durum) had an important centre of diversification in Ethiopia, while Chromosome 4 was chosen since it is one of the chromosomes where QTLs for drought tolerance have been detected. The primers were selected to have, ideally, a locus every 5 cM. The results indicate the presence of 2 selective sweeps in the regions with drier environments. One, as expected, is located in a telomeric position of the short arm, in a position similar to the one where previous QTL for drought tolerance was found. But the other is localized in a new position on the 4AL.

These data indicate the existing of high selective pressure in the particular climatic conditions of the Ethiopian territory which is from always a natural laboratory for the study of the genetic variability and for the selection of genotypes with particular climatic adaptations.
COMPARATIVE PROTEOMIC ANALYSIS OF HEAT STRESS ON DURUM WHEAT GRAIN PROTEINS

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heat stress, two-dimensional gel electrophoresis, durum wheat, proteome, wheat kernel proteins

Durum wheat is one of the most important crops grown in our Country, in particular Central and South Italy. In our climate, grain filling occurs between April and May, when sudden temperature raisings may take place. High temperature during grain filling had already been recognized to cause a deviation of expected properties and quality characteristics of bread wheat doughs. This was a consequence of differential accumulation of grain proteins, that resulted in an alteration of their ratios, that, in their 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 so-called 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 to two thermal regimes (heat stress vs. control), by producing four biological replicas for each treatment.

One dimensional electrophoretic analyses (APAGE and SDS-PAGE) were performed on gliadins and glutenins, in order to calculate the ratios between the different protein fractions. The ratio between each gliadin class (a/b, g, w) and total gliadins, as well as the ratio between high and low molecular weight glutenin subunits (HMW-GS/LMW-GS) have been calculated.

Two-dimensional electrophoresis (IEF vs SDS-PAGE) was carried out on the metabolic (non-prolamin) and total gluten (prolamin) fractions, separately. IPG strips (17 cm long) in the pH range 3-10 were used to perform three different technical replicas for each biological replica for each protein fraction, for a total of 48 2D gels. Spots were revealed with Coomassie Brilliant Blue (CBB) and analyzed with the software Progenesis SameSpots (Nonlinear Dynamics, UK), in order to identify differentially expressed polypeptides between heat stressed and control plants.

Results obtained so far indicate that gliadins were overexpressed in the heat stressed samples, in particular w-gliadins, and this was in agreement with what already found in bread wheat. The HMW-GS/LMW-GS ratio was not affected, whereas minor protein spots found in the total gluten fraction as analysed by 2D comparison, resulted differentially expressed. Although mass spectrometry analysis has not been perfomed yet on such spots, on the basis of their mobility we hypothesize that they actually correspond to non-gluten proteins, that are contaminants of the gluten
fraction, either because they are accidentally co-purified, or because they are covalently linked to gluten proteins. Analysis of the non-prolamin fraction, in fact, shows differentially expressed spots that will also be characterized by mass spectrometry.
MENDELIZATION OF HETEROISTIC QTL BY MEANS OF HETEROGENEOUS INBRED FAMILIES AND NEAR ISOCENIC LINES IN MAIZE

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heterosis, QTL, maize, HIF, NIL

Heterosis is widely exploited for crop improvement and breeding, but its genetic bases are still elusive. However, integration of biometrical and biomolecular tools has revealed as a successful strategy for the fine genetic dissection of complex traits. In this perspective, a classical genetic analysis and a combined QTL analysis were previously applied on maize materials originated from the single cross B73 x H99. Level of heterosis for several agronomic traits and the underlying genetic effects (allelic and non-allelic interactions) were evaluated, along with the relationship between level of heterozygosity and phenotypic performance. A number of QTL for heterosis were detected, which suggest that hybrid vigor is mainly due to allelic interaction, especially overdominance and/or pseudo-overdominance, while non-allelic interaction being of minor importance. Based on results previously obtained, we undertook an introgression program aimed at the Mendelization of the six most relevant heterotic QTL into Near Isogenic Lines (NILs). The adopted strategy allowed obtaining NILs within only three to four cycles of controlled crosses starting Heterogeneous Inbred Families (HIF) of nearly-isogenic individuals, i.e. from RIL-F₅ that were selected by molecular markers for being heterozygous at regions harboring interesting QTL. Each HIF is isogenic at the majority of loci in the genome and NILs differing for markers linked to QTL of interest can be produced from segregating families. Where possible, two different NIL sets were produced from two different HIFs for each QTL, in order to allow discriminating the influence of the genetic architecture on the effect of single QTL. Each NIL set, produced by marker assisted selection, consists of two single seed descent lines each carrying the QTL region in homozygosis for one or the other parental line (i.e. B73 and H99). In the process of obtaining NILs, HIFs were preliminarily evaluated for grain yield and other traits to validate two major heterotic QTL. NIL sets obtained for all the six QTL have been then test-crossed (TC) both to B73 and H99 for a further field evaluation of additive and non-additive QTL effects. In both advanced HIF and TC analyses,
heterozygotes proved to have an advantage over the mean of homozygotes for most of the introgressed QTL, consistently with the original QTL analysis. NILs pairs were also crossed to each other to obtain heterozygous pseudo-F$_1$, for future fine mapping and possibly QTL cloning. Furthermore, we are carrying on an extended testcross program including six additional inbred lines for evaluating the consistency of QTL effects in different unrelated genetic backgrounds.
THE PIN FAMILY OF AUXIN TRANSPORT GENES IN ZEA MAYS:
LOOKING FOR PINS IN A STRAW-STACK

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auxin, PIN-formed, polar auxin transport, Zea mays

Genetic approaches in Arabidopsis thaliana identified candidate genes involved in auxin transport, among them permease-like AUX1, PIN proteins and homologs of human multiple drug resistance transporters (MDR-PGPs). The PIN-FORMED (PIN) protein family is a group of plant specific transmembrane proteins belonging to the “mem_trans” PFAM’s group which is one of the several groups of secondary transporters. PINs have been shown to play a rate-limiting role in the catalysis of efflux of auxin from cells, and their asymmetrical cellular localization determines the direction of cell-to-cell auxin flow.

There are eight PIN genes in the genome of Arabidopsis: they encode proteins of between 351 (AtPIN5) and 647 (AtPIN2) amino acids in length. Their relatively high amino acid identity suggests that all PIN genes in Arabidopsis (and in all higher plants) diverged from a single ancestral sequences. Genes homologous to the Arabidopsis PINs are present in genomes throughout the plant kingdom, from Physcomitrella patens to all vascular plants. Dicot and monocot plants show significant changes in the number and the structure of PIN genes. However, in dicot plants, the phylogenetic structure of the family has been broadly conserved: both Medicago truncatula and Solanum tuberosum contain at least five PIN sequences that show similarity to one of the eight PINs of Arabidopsis. Additional evidence for such a broadly conserved phylogenetic structure in the PIN genes comes from Glycine max and Brassica juncea. In contrast to dicots, the wider PIN family of the monocots has a more divergent phylogenetic structure, with two or three genes homologous to one Arabidopsis PINs. Triticum aestivum and Oryza sativa present respectively three and two closely related PIN1 genes. On the other end TaPIN9 and OsPIN9 do not clusterize with any dicot sequence, suggesting the presence of at least one monocot-specific PIN protein.

Zea mays present a wider and more divergent PIN family if compared with the wheat and rice ones. Recently we identified three orthologs of AtPIN1, called ZmPIN1a, ZmPIN1b and ZmPIN1c. The amino acid identity between the putative maize proteins is 85%, while the AtPIN1 protein exhibits an amino acid identity around 70% with maize PIN1s. In addition RT-PCR experiments let us the identification of three different ZmPIN1b splicing variants. Their role in maize development is still under analysis. To investigate the structural diversity of the maize PIN protein family we searched for PIN-like sequence in public database using Arabidopsis and rice PINs as queries. The program BLAST found at least ten complete sequences, which were aligned with ClustalW algorithm. The alignment was used to construct a neighbor-joining phylogenetic tree, including PIN sequences from wheat, rice and Arabidopsis. We named maize sequences according to which cluster of the Arabidopsis PIN family they belong. The preliminary results confirm the widening of maize PIN family, that include at least two genes closely related to AtPIN2 and two putative orthologues to AtPIN4. Moreover, this analysis shows that the three ZmPIN1 sequences fall into the
same cluster together with the orthologous PIN1 proteins of rice, Arabidopsis, and wheat, confirming our previous results.
IDENTIFICATION OF GENES AFFECTING KERNEL PHENOTYPE VIA OPAQUE MUTANTS AND TRANSCRIPTOMIC TECHNOLOGY

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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 (Mc) 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 A69Ywt, A69Yo2, A69Yo7, and A69Yo2o7 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 A69Ywt and A69Yo2 genotypes. Conversely, the A69Ywt and A69Yo7 genotypes show less evident differences in expression levels. The A69Yo2o7 double mutant exhibits differences in expression patterns resembling those obtained for the A69Yo2 genotype. A plot of A69Yo2 vs. A69Yo7 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 A69Yo7 background, whereas 759 ESTs showed a reduced expression pattern in A69Yo2o7. Up-regulated expression profiles were found for 3.23% of the ESTs considered. One hundred and thirteen up-regulated ESTs were identified in the A69Yo2, 26 in the A69Yo7, and 86 in an A69Yo2o7 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.
THE NUCLEAR GENE EMPTY PERICARP4 ENCODES A PENTATRICOPEPTIDE REPEAT PROTEIN REGULATING THE EXPRESSION OF A SMALL GROUP OF MITOCHONDRIAL GENES IN ZEA MAYS ENDOSPERM


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maize, emp4, pentatricopeptide repeat protein, mitochondrial genes, endosperm

PPR proteins are characterized by multiple repeats of a degenerate 35–amino acid motif containing a distribution of hydrophobic and hydrophilic residues. The repeats are usually present as tandem arrays, with an average number of 12 motifs per polypeptide. The sequences of these motifs are very degenerated but the suggested structure seems conserved and consists in two alpha helices. In most PPR proteins, the N terminus contains organelle-targeting signals that show a poor degree of sequence conservation. By contrast, some PPR proteins show a high degree of amino acid conservation at the C terminus, which is used to classify plant PPR proteins into reduced family groups. The empty pericarp4 (emp4) gene has been cloned in maize by transposon tagging. Sequence analyses revealed that emp4 encodes a 614–aminoacid protein that is highly homologous with the PPR class of proteins. EMP4 contains nine PPR motifs preceded by a short sequence showing partial homology with the 31–amino acid PPR like short motif previously described (Lurin et al. Plant Cell 16, 2089-2103, 2004). Interestingly, the domain found at the N terminus of EMP4 was conserved in a wide range of plant PPR proteins, whereas the domain identified at the C-terminal region of EMP4 was found in only three other proteins: two Arabidopsis thaliana PPR proteins, At3g49730 and At5g65820 respectively and one predicted rice (Oryza sativa) protein AC135956.

PPR genes in plants are thought to encode RNA binding proteins with essential roles in organelles. Because EMP4 appeared to target mitochondria, we investigated whether a mutation in emp4 had any effect on gene expression in this organelle. Microarray analysis of mitochondrial gene expression in immature wild-type and emp4-1 mutant endosperms revealed a considerable reduction in gene expression for only a small subset of mitochondrial genes in emp4-1 endosperms. Data were confirmed by RNA gel blot hybridization analysis where we found lower expression of both rps2A/rps2B and rps3/rpl16 and a drastic reduction in mtub (orfX) transcript in emp4-1 mutant endosperms compared with sibling wild-type endosperms (Gutiérrez-Marcos et al. Plant Cell19: 196-210, 2007).

Despite the growing number of genetics studies and in vitro experiments, the nature of the in vivo target of the vast majority of PPR proteins has not yet been identified and the mechanisms of action of PPR proteins are still poorly understood. emp4 might be involved in controlling the level
of expression of these mitochondrial genes or in promoting their transcripts’ stability. At present our work is aimed at describing the role of \textit{emp4} in different plant tissues and detecting the molecular partners of EMP4. To this aim biochemical as well as genetic approaches will be undertaken.
ELUCIDATING THE ROLE OF ZmPIN1 GENES DURING MAIZE KERNEL DEVELOPMENT

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embryogenesis, endosperm, PIN-formed, polar auxin trasport, Zea mays

In Angiosperms the seed is the outcome of a double fertilization event, a process leading to the formation of the embryo and the endosperm. Proper seed development requires the co-ordinated expression of embryo and endosperm genes and relies on the interaction between the two seed compartments and between the seed and the maternal tissues. The large reservoir of auxin conjugates deposited into the developing maize endosperm, which provides a continuous environment for the developing embryos, has been suggested to be involved in these interactions, leading to polar embryo development. In Arabidopsis thaliana an apical-basal auxin gradient, established by PIN7, triggers the specification of apical embryo structures, whereas the subsequent PIN1 polar localization reorganizes the auxin gradient to specify the basal root pole. To verify whether this model also applies for monocotyledonous species, in which embryos develop with a more complex architecture and endosperm persists at later stages of seed development, we investigated on the behavior of ZmPIN1 genes and auxin accumulation patterns during Zea mays kernel development.

We identified ZmPIN1c, a novel putative ortholog of AtPIN1 in maize. This gene, encodes a putative protein of 597 amino acids that shows more than 80% of amino acid identity with ZmPIN1a and ZmPIN1b, the others two members of the ZmPIN1 family. Real time RT-PCR experiments demonstrated that ZmPIN1 genes show differential expression patterns during kernel formation. In situ hybridization assays with ZmPIN1 specific probes and immunolocalization assays using an anti-AtPIN1 antibody revealed different localization of PIN1 transcripts and proteins in developing kernels. During the differentiation of endosperm four different cellular domains (basal transfer layer, embryo surrounding region, starchy endosperm and aleurone), ZmPIN1a and ZmPIN1c localized in the basal transfer cells layer (BETL) and in the embryo-surrounding region (ESR), but ZmPIN1 proteins are not polarized both in BETL and ESR domains. During embryogenesis ZmPIN1 genes are expressed in the apical region of the proembryo, in the scutellum during the transition and the coleoptilar stage and in the shoot apex and root from L1 to L5 stages. The embryonic SAM is characterized by a central group of cells presenting a polarized PIN1 in a way that suggests auxin fluxes spreading at 360°. The protein also marks leaf primordia and L1 layer at the level of the incipient primordium. In the embryonic root the antibody suggests acropetal auxin fluxes directed towards the RAM. To better describe the auxin fluxes during kernel development we performed immunolocalization experiments using an anti-IAA antibody. Our data showed that auxin is present both in the maternal tissues of the seed and at higher level in specialized tissues of the endosperm: aleurone, transfer layer and embryo surrounding region.
defective endosperm-B18 maize mutant, that shows reduced levels of IAA in the endosperm leading to a reduced dry matter accumulation also showed altered expression of PIN1 genes and defects in differentiation of the transfer cell layer.
STRUCTURAL CHARACTERISATION OF GENES ENCODING PROTEINS OF THE PDI FAMILY IN WHEAT

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Protein Disulfide Isomerase (PDI) gene family, wheat, gene structure, expression analysis

The PDI (Protein Disulfide Isomerase) family includes several genes whose products are responsible for diversified metabolic functions, the proteins differ also for number and position of the active thioredoxin-like sites, for presence/absence of other domains and of the KDEL signal of retention in the endoplasmic reticulum. In plants the PDI family includes eight different phylogenetic classes. Isolation and characterization in wheat of the three homoeologous gene sequences encoding classical PDI (TaPDIL1-1) and of their promoter sequences have been reported previously. Some studies of molecular characterization, expression analysis and cell localisation in rice and maize have suggested the involvement of the classical PDI in the assemblage and deposition of storage proteins in these species. In wheat the likely involvement of the classical PDI, as well as the potential participation of PDI-like proteins, in the storage protein folding and in formation of high molecular weight protein aggregates makes their study particularly interesting. Our goal is the characterization of the complexity and diversity of the PDI gene family in wheat. A cross search using PDI-like sequences of rice in the wheat EST databases “TIGR wheat gene index” and “HarvEST Wheat” identified nine sequences coding for PDI-like proteins in wheat, whose full length cDNAs have been cloned. Phylogenetic analysis allowed the assignment of the ten PDI and PDI-like sequences of wheat to the eight phylogenetic groups identified in plants. Thus at least one gene has been cloned for each phylogenetic group. The search for conserved motives in the deduced amino acid sequences of the nine isolated genes, by comparison with sequences in different protein data bases, revealed a high level of structural similarity between the proteins encoded by genes belonging to the same phylogenetic group. The comparison of the genomic organisations of three wheat PDI-like genes (TaPDIL2-1, TaPDIL4-1 e TaPDIL5-1) with their orthologous of rice and Arabidopsis showed a high level of conservation of their structural features (exon/intron structure, exon length and position of the active sites) among members of the same phylogenetic group. Most likely such conservation reflects the essential functional role of their encoded proteins. The chromosome location of the genes encoding two wheat PDI-like proteins (TaPDIL4-1 and TaPDIL5-1) was determined through Southern analyses of DNA extracted from nulli-tetrasomic and ditelosomic lines of Chinese Spring. The three homoeologous gene sequences encoding TaPDIL4-1 were located in the short arm of the group 1 chromosomes, those encoding TaPDIL5-1 in the long arm of the group 5 chromosomes. Northern analysis of TaPDIL2-1, TaPDIL4-1, TaPDIL5-1 and TaPDL1-1 detected different expression patterns in the analysed tissues. Further studies will be necessary to complete the molecular characterisation of this multigenic family in wheat. The structural characterisation and detailed analysis of expression patterns will be extended to the remaining six genes encoding PDI-like proteins. An exhaustive knowledge of the structural features and regulation of the PDI family genes will be useful to design the most suitable strategies
for their functional characterization, in particular for the silencing of single genes or of gene groups through the RNA interference (RNAi) technology. The effect on the characteristics of seed storage proteins produced by the progressive knock-out of PDI and PDI-like genes will allow the understanding of their role in the formation of protein aggregates and will highlight possible functional redundancies.
QTL MAPPING FOR ROOT ARCHITECTURE TRAITS IN MAIZE USING A GASPE’ FLINT x B73 INTROGRESSION LIBRARY

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quantitative trait loci, root development, seminal root, drought stress

The genetic control of root architecture in maize was studied using an introgression library (IL) developed from the cross B73 x Gaspé Flint. The IL is constituted of 72 lines developed by five cycles of marker-assisted backcross using B73 and Gaspé Flint as recurrent and donor parent, respectively. Each IL line retains 30-40 cM of Gaspé Flint genome and an estimated 70% of Gaspé Flint genome appears represented within the collection. Based on trait differences between the donor and the recipient genotypes, the IL collection will serve as a permanent source of nearly isogenic material for QTL analysis and cloning for many morpho-physiological traits such as root and plant architectures, flowering time, yield and yield components, etc.

IL lines were screened using a simple “paper-roll” method (Hetz et al., 1996) to search for QTLs controlling root traits at the seminal stage of development (e.g. length and dry weight of the primary and seminal roots, number of seminal roots). The same set of ILs were also grown in pots to study root traits at a later stage of development. Particular striking differences were observed between the two parental lines and among the IL lines for the number of seminal roots developing from the scutellar node. B73 produced an average of 2.8 seminal roots per plant while Gaspé did not show any seminal root. Within the IL, we found a few IL lines showing a Gaspé-like phenotype, implying that the QTLs controlling this trait are localized on the introgressions carried by these lines. A summary of the data on QTLs for root architecture at the seedling level will be presented.
AN INTEGRATED GENETIC AND PHYSICAL MAP OF GAMETOPHYTE FACTOR 1 (Ga1) GENE IN MAIZE

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Ga1, molecular markers, integrated map, synteny, positional cloning

Ga1 (Gametophyte Factor 1) is a gametophyte mutant involved in the phenomenon of cross-sterility. Such mutant is widely present in many popcorn inbred lines. The presence of the dominant allele Ga1s in the sporophyte prevents fertilization by pollen carrying the recessive allele ga1. The gene was discovered in 1923 and roughly mapped on maize chromosome 4, exploiting its loose linkage with another mutant, sugary 1 (Su1) (House and Nelson, 1958). However, its position was never determined in greater detail, and up to now this gene has never been precisely mapped on public genetic maps.

Our present aim is the isolation of Ga1 through a positional cloning approach. First, we generated a large segregating population of around 3000 individuals, phenotypically and molecularly characterized.

Various approaches to identify molecular markers in the region of interest have been adopted; search on public maize databases has been performed, followed by the exploitation of rice-maize synteny.

We then integrated data from physical and genetic maps. We placed additional molecular markers obtained from maize ESTs, genomic and BAC-ends sequences derived from clones sequencing in order to further reduce the region containing Ga1.

Analysis of the public physical map (http://www.genome.arizona.edu) has led to the identification of a single BAC contig.

We subsequently created a high-resolution map of the chromosomal region of interest, delimiting a genetic region of 1.8 cM encompassing Ga1, and we could thus link our genetic map to the physical one.

The exploitation of rice-maize synteny has been pursued to identify a region on rice chromosome 11, syntenous to the maize interval flanking Ga1 (www.gramene.org).

At the present stage we can search for new sequences in this delimited rice region, orthologous to the maize region surrounding Ga1. Furthermore, we will attempt to restrict the physical region to few clones in order to sequence them and search for potential candidate genes.

In any case, we will need a genomic library of the line Ga1/Ga1 in the final steps of positional cloning, as the public physical map was obtained from inbred line ga1/ga1 (B73). Indeed, it is well known that genetic diversity among inbred lines in maize could be surprisingly wide.
GENETIC ANALYSIS OF THE SHOOT APICAL MERISTEM IN MAIZE

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Shoot Apical Meristem, maize, shootmeristemless mutant

The Shoot Apical Meristem is established during embryogenesis and is a key event in the plant development; it is the source of stem cell as well as the site of organ formation. Because of the importance of SAM function in plant development, the mechanisms of formation, maintenance and function are crucial questions in plant developmental biology.

A useful tool to investigate the SAM is the analysis of mutants impaired in its organization, like the sml and dgr maize mutants described below.

The sml (shotmeristemless) gene is a recessive mutation affecting shoot apical meristem maintenance and lateral organ formation. Its introgression 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 gene lies on the long arm of chromosome 10. Its position has been defined by B-A translocations mapping followed by the linkage analysis with visible as well as molecular markers.

The dgr phenotype displays a variety of plant and leaf abnormalities and the severity of leaf defects may vary widely within a single mutant plant, including half leaf, thread leaf and narrow leaf phenotype.

The inflorescence is also affected exhibiting male flower sterility, ears often developing secondary ears in husk leaf axils at the base of the main ear and female flower showing extra silks.

This phenotype can be due to a defective dgr SAM. The morphological analysis and the detection of the shoot marker gene expression domain in mutant apices will reveal its organization.

The histological analysis of mutant seedlings reveals that in the dgr shoot the L1 outer layer cell shape is less regular than in the wild-type. The L1 layer plays a key role in the shoot being necessary for maintenance of indeterminacy in the underlying meristem layers, and for the specification of the adaxial fate.

In maize adaxial/abaxial leaf polarity is established by an abaxial gradient of microRNA166 which spatially restricts the expression domain of HD-ZIPIII transcription factors that specify adaxial fate.

Recessive mutation in lbl1 lead to a variable abaxialization of leaves, showing a phenotype very similar to the dgr plant; Lbl1 gene is involved in the biogenesis of trans-acting small interfering RNAs that acts on the adaxial site of developing leaves and demarcates the domain of hd-zipIII and miR166 accumulation.

The analysis of the dgr leaf polarity and the double mutant sml-lbl1 will define the relationship between these genes.
MOLECULAR AND GENETIC CHARACTERIZATION OF FUSED LEAVES A GENE AFFECTING SHOOT APEX ORGANIZATION AND GLOSSY DEPOSITION IN MAIZE

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The fused leaves (fdl) mutant was identified in an active Suppressor-mutator (Spm) line. It behaves as a monogenic recessive mutant and the analysis of homozygous fdl plants indicates that the mutant phenotype is confined to early stages of plant development. Mutant seedlings are retarded in their germination and growth in comparison with wild-type relatives and exhibit distinct features, such as a thicker coleoptile and presence of curly and glossy juvenile leaves. Furthermore, the opening of the mutant coleoptile occurs with an irregular lateral fracture in contrast to the clear-cut hole that is formed in the wild-type.

The co-segregation analysis has revealed a link between the mutant phenotype and a Restriction Fragment Length Polymorphism hybridizing with an Spm probe and has allowed the isolation of small genomic region of 78 bp flanking the transposon insertion. This sequence exhibits a complete homology with the Zea mays GSS contig (ZmGSSStuc11-12-04.5024.1) that contains an EST sequence (AW267377).

Primers were designed on the basis of these sequences and they were used with Spm-specific primers in the mutant and wild-type genomic DNA comparison by means of PCR approach. The exact position as well as the orientation of the Spm element in the mutant region have been determined. In addition, sequence-specific primers were designed and used in rapid amplification of cDNA ends (RACE) in order to generate a full-length cDNA. Computational analysis has revealed that the putative ORF derived from the cloned sequence contains a Myb R2R3 motif.

Semi-quantitative RT-PCR has also been performed on the germinating seedling at different times, and has revealed a higher transcript level at the first stages of seedling development. The transcript was also detected in silk, anthers and maternal tissue.

To confirm that the cloned sequence corresponds to the fdl gene, different approaches are presently undertaken. The linkage between the Myb containing genomic fragment and the fdl phenotype has been analyzed on a large scale in segregating F2 populations by means of PCR approach in which a sequence specific primer is used with an Spm primer. A targeted mutagenesis approach has also been undertaken for the isolation of new alleles at the fdl locus. To this aim a Mutator active line (MuDR) has been crossed as pollen donor with homozygous fdl plants. The new alleles will constitute a proof for the gene identity.
TOWARD A MUTATIONAL SATURATION OF THE PATHWAY LEADING TO VIVIPARY IN MAIZE


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vivipary, abscisic acid

Vivipary in maize is the diagnostic phenotype of mutants impaired in the biosynthesis or response to abscisic acid (ABA). Of the 15 viviparous (vp) mutants so far described 12 control specific steps in ABA biosynthesis, two are response mutants and one is still undefined in its role. We have analyzed a collection of 26 independent vp isolates with the intent of determining the degree of mutational saturation that has been so far reached. A complementation test among the new vp accessions and those previously described led to the identification of six alleles of vp1, five of vp5, one each of vp7 and vp9 and seven of vp10. Of the remaining six, three define new genes (rea, vp404* and vp366*) while the other three (vp394*, vp103*, vp110*) are still under analysis. These results suggest that we are approaching the mutation saturation point of the ABA biosynthesis and signalling pathway. All mutants so far tested (17 in total) except rea show a decrease in ABA content and sensitivity to exogenous ABA, an observation in agreement with a role of these genes in the biosynthesis of the phytohormone, but unexpected for those allelic to vp1. As to the only two mutants (vp1 and rea) whose endogenous ABA content is not impaired, the reduction in sensitivity of the double mutant compared to the single ones suggest that the two genes control separate pathways in the ABA signal transduction. Some of the mutants in this collection have a characteristic incipient vivipary that allows the embryo of the mature dry seed to resume germination, an event not observable in viviparous seeds. By exploiting this feature it is possible to infer, through a germination test, if the mutant has been impaired in the acquisition of desiccation tolerance. The results obtained have been confirmed by testing the germination rescue of immature mutant embryos subjected to premature desiccation. This information provides the starting point for the dissection of the genetic basis of desiccation tolerance.
ALPHA-TRYPTOPHAN SYNTHASE-LIKE OF ISATIS TINCTORIA: GENE CLONING AND EXPRESSION

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Arabidopsis thaliana, indigo, indole, Isatis tinctoria, tryptophan synthase α-subunit

Indole producing reaction is a crux in the regulation of metabolite flow through the pathways and the coordination of primary and secondary product biosynthesis in plants. Indole is yielded transiently from indole-3-glycerol phosphate and immediately condensed with serine to give tryptophan, by the enzyme tryptophan synthase (TS). There is evidence that plant TS, like the bacterial complex, functions as an a b heteromer. In few species, e.g. maize, are known enzymes, related with the TS α-subunit (TSA), able to catalyse reaction producing indole, which is free to enter in secondary metabolite pathways. In this contest, we searched for TSA and TSA related genes in Isatis tinctoria, a species producing the natural blue dye indigo. The It-TSA cDNA and the full-length exons/introns genomic region were isolated. The phylogenetic analysis indicates that It-TSA is closer related to Arabidopsis thaliana At-T14E10.210 TSA (95.7% identity at the amino acid level) with respect to A. thaliana At-T10P11.21 TSA1-like (63%), Zea mays indole-3-glycerol phosphate lyase (54%), Z. mays TSA-like (53%), and Z. mays indole synthase (50%). To examine the involvement of It-TSA in the biosynthesis of secondary metabolism compounds, It-TSA expression was tested in seedling grown under different light conditions. Semi-quantitative RT-PCR performed showed an increase in the steady-state level of It-TSA mRNA, paralleled by an increase of indigo and its precursor isatan B. Our results appear to indicate an involvement for It-TSA in indigo precursor synthesis and/or tryptophan biosynthesis.
REFERENCE GENES SELECTION FOR GENE EXPRESSION STUDIES IN BARLEY AND GRAPE

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reference genes, barley, grape, RT qPCR

Advanced gene expression analysis methods, such as microarray and real-time RT PCR, require efficient normalization approaches to be really informative. Normalization means to adjust expression data for effects arising from the lab technology applied rather than from real biological differences among samples. Inhibitory factors in the tissues, loading errors, integrity of the RNA are just some of the parameters to be taken into account during the quantification process. Therefore, expression results are now normalized against a set of reference genes that should be expressed in an unchanging fashion regardless of experimental conditions. However, in plants, there are just few examples of studies specifically concerned with housekeeping gene expression analysis and very often they are focused on validation of a list of literature based reference genes in the experimental condition of interest.

In our work, an EST based approach has been developed to identify novel candidate housekeeping genes in two plants of great economic concern, like barley (Faccioli et al, 2007) and grape, for which EST databases are publicly available. A set of reference genes has been identified for both species, their expression stabilities have been measured in different experimental conditions through RT qPCR analyses and the results obtained have been evaluated with dedicated bioinformatics tools.

Reference
A PARALLEL PIGMENT AND TRANSCRIPTOMIC ANALYSIS OF FOUR BARLEY ALBINA AND XANTHA MUTANTS REVEALED THE COMPLEX NETWORK OF THE CHLOROPLAST-DEPENDENT METABOLISM

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plastid biogenesis, barley, oligo-array, chlorophyll biosynthesis

We investigated four *albina* and *xantha* barley mutants characterized by a block in sequential steps of the chloroplast biogenesis and the corresponding wild type (WT) with the Affymetrix Barley1 GeneChip® (about 22,000 probe sets) to assess the variations of gene expression associated with chloroplast development. Chloroplast development is intimately interconnected to carotenoid and chlorophyll biosynthesis and we have been studied thoroughly these pathways analyzing all intermediates of the pigment biosynthetic pathways by HPLC analysis. The availability of a genome wide gene expression data set and of a detailed analysis of pigment contents from the same samples has allowed us to make a parallel comparison of transcriptomic and metabolomic data. At transcriptional level the alb-e16 mutant is characterized by a clear down-regulation of the gene coding for one of the three subunits of Mg-chelatase. The repression of chlH in alb-e16 is associated with an over-accumulation of Proto in ALA-fed plants, therefore both metabolic and gene expression data are consistent with a block of the chlorophyll biosynthetic pathway before Mg-proto biosynthesis. The alb-e16 also showed a down-regulation of the gun4 homologous barley gene coding for the ChlH/Mg-ProtoIX-binding protein.

The key feature of the alb-f17 mutant was the down-regulation of the PorA gene encoding one of the two subunits of the POR enzyme, in presence of a normal or an up-regulated expression of Mg-chelatase genes. The repression of PorA in alb-f17 was associated with an over-accumulation of Mg-proto in ALA-fed plants, therefore both metabolic and gene expression data are consistent with a block of the chlorophyll biosynthetic pathway before Chlide biosynthesis. The down-regulation of PorA is also associated with an up-regulation of gene coding for OEP16, a component of the POR-A Pchlide-dependent translocon complex both in alb-e16 and alb-f17 mutants. An additional feature of alb-f17 was represented by the over-expression of several genes involved in the phytochrome and in the phytochrome-dependent pathways. The expression profile of genes known for their involvement in plastid-to nucleus signaling were used to search for other genes co-expressed across all samples.
Although, co-expressed genes are not necessarily co-regulated, these analysis provide additional evidences on a chloroplast-dependent covariation of large sets of nuclear genes and suggest that different chloroplast-nuclear signals might control different sets of genes.
AN INTEGRATED GENOTYPING-PHENOTYPING CHARACTERISATION OF ITALIAN RICE GERMPLASM


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rice, genomics, breeding, phylogenetic analysis

This study was undertaken to investigate the correlation patterns between phylogenetic groups existing in the Italian rice germplasm and agronomic traits with specific reference to biotic stress resistance. A total of 180 Italian rice cultivars representing all varieties cultivated in at least one thousand hectares and 44 exotic varieties which either entered the breeding programs in Italy or represented reference varieties at international level. Genomic DNA was purified from young leaves and a biorepository in bar-coded 96-well plates was created and made available to the scientific community. Genotyping analysis was carried out using 24 SSR markers distributed throughout the rice genome. The clustering analysis performed using the DARWIN program identified eight phylogenetic groups, of which four major and four minor ones. Phenotyping for blast resistance was carried out in controlled conditions with three-week old plants and three M. grisea strains representing the existing pathogen biodiversity in Italy and in Europe. Correlation studies genotype-phenotype were carried out interpolating available data for seed biometry, plant height, amylose content, and the generated data for blast resistance to the identified phylogenetic groups. These analyses revealed the existence of phylogenetic groups that were recognisable for their origin and agronomic traits. The accumulated information is valuable to ongoing breeding programs at national level to direct them towards the best germplasm combinations to combine valuable traits in elite varieties using marker-assisted selection strategies.
THE RICE *OSMYB4* GENE AUTOREGULATES ITS OWN EXPRESSION

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*Osmyb4*, stress tolerance, transgenic rice, orthologous genes, autoregulation

The rice *Osmyb4* gene, isolated from a coleoptile cDNA library and encoding a Myb transcription factor, is involved in tolerance/resistance to several abiotic and biotic stresses. Our previous data indicate that *Osmyb4* ectopic expression in several species activates stress response pathways in both mono- and dicotyledonous, the specificity of action depending on the host plant. These results suggest that *Osmyb4* represents a crucial knot in a stress signalling network conserved among species.

The analysis of a phylogenetic tree revealed that *Osmyb4* belongs to a small Myb subfamily of three members and analogous families are present in several species. Literature data and our findings about the expression of these genes in different stress conditions indicate that what is maintained is the function of the family as a whole rather than the function of the single genes.

Unexpectedly, transgenic rice lines with different expression levels of the exogenous *Osmyb4* gene did not seem to show any differences in morphological phenotype, stress tolerance and metabolic profile with respect to wild-type plants. An expression analysis with specific oligonucleotides able to discriminate between the endogenous and the exogenous *Osmyb4* forms suggested the ability of *Osmyb4* to autoregulate its own expression. In fact, at low levels of the endogenous transcript (vegetative stage), the presence of the exogenous *Osmyb4* mRNA increased the expression of the endogenous form, whereas at high levels of the endogenous transcript (reproductive stage), the exogenous mRNA caused a proportional decrease in the level of the endogenous one. Such a compensatory mechanism may explain the lack of a Myb4-driven evident effect on transgenic plants phenotype.

We actually demonstrated the ability of Myb4 to inhibit the transcription activity of its own promoter through transient expression assays in tobacco protoplasts. This ability must depend on cis-acting elements present on the *Osmyb4* promoter, since in the same conditions Myb4 is able to transactivate other promoters (PAL2 and Des9).

*In silico* analysis of the *Osmyb4* and its putative paralogous and orthologous gene promoters highlighted the presence of several cis elements described as responsive to abiotic and biotic stresses that may be responsible for the stress induction of these genes. Moreover, the presence of some Myb binding sites suggested that also the expression of Myb4 paralogous and orthologous genes may undergo a mechanism of auto- or cross-regulation. This hypothesis seems to be supported by the negative effect of the Myb4 expression in transgenic Arabidopsis on the expression of the endogenous *Atmyb14* gene.

The presence of a fine autoregulation mechanism is in agreement with the high complexity of the Myb4-activated pathway. It suggests that similar mechanisms may regulate the level of the expression of other upstream acting genes as well. Being aware of this possibility is crucial for successful application of transgenic approaches to crop improvement.
CHARACTERIZATION OF APOSTART MEMBERS/ALLELES IN *POA PRATENSIS* L.

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*apomixis, P. pratensis, Real time-PCR, in situ hybridization*

Poa pratensis L. (Kentucky bluegrass) has an extremely versatile reproductive system, the main pathways being aposporous apomixis and sexual outcrossing. Aposporous apomixis is functionally composed of two processes: apospory and parthenogenesis. In *P. pratensis*, apospory involves the development of embryo sacs from somatic cells that differentiate into the nucellus. If unreduced polar nuclei positioned centrally fuse with a sperm cell released from the pollen tube (pseudogamy), the unreduced egg within the embryo sac can develop autonomously through parthenogenesis to form viable apomictic seeds. Cyto-histological investigations of parthenogenesis and apospory in an F1 population resulting from a cross between a sexual (non-aposporic and non-parthenogenetic) and an apomictic (aposporic and parthenogenetic) parents allowed us to isolate 5 genotypes recombinant for one of the two apomictic features (aposporic and non-parthenogenetic or non-aposporic and parthenogenetic). These recombinants could be very useful to isolate the genetic determinants responsible for, or involved in, the single components of apomixis in *P. pratensis*.

The expression pattern of 11 APOSTART members/alleles was investigated using mRNA isolated from inflorescences at 5 developmental stages of *P. pratensis* of sexual, apomictic and recombinant genotypes through Real-time PCR.

Moreover, the spatial distribution of APOSTART transcripts within reproducing organs of apomictic, sexual and recombinant genotypes of *P. pratensis* was determined by an high number of independent in situ hybridization experiments.

The genomic organization and characterization through temporal and spatial expression analysis of transcripts in reproductive tissues are reported and discussed.
GENOMIC AND CDNA LIBRARIES FOR ISOLATION OF GENES POTENTIALLY INVOLVED IN APOMIXIS IN POA PRATENSIS L.

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apomixis, genomic libraries, cDNA libraries, promoter, P. pratensis

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. The isolation of specific genes is crucial for understanding the molecular-genetics of complex traits, such as apomixis. Even if new cloning strategies 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. Among several choices, we have applied a cDNA-AFLP technique to staged inflorescences of P. pratensis with the aim of cloning genes putatively involved in apomixis and have identified several ESTs differentially expressed between apomictic and sexual genotypes. The first objective of this work was to isolate the full-length genes of the most promising ESTs and to do so, we created 2 full-length cDNA libraries (1 from an apomictic and 1 from a sexual genotypes) and isolated the full-length of 6 genes (APOSTART, PpSERK, PpAPK, PpARM, PpRAB and PpMOB1). In particular, several APOSTART members showed to be flower-specific and so our aim was to isolate the relative promoter(s). Genome walking strategies failed since it seems that in the 5’-end of the gene resides a retrotransposon region which did not allowed the walking upstream the gene. We, then, decided to create 3 genomic libraries (2 from apomictic and 1 from a sexual genotypes) based on cosmid vectors. These libraries were screened for APOSTART as well for the other genes of interest reported above. The isolation and bioinformatic characterization of cDNA and genomic clones is reported and discussed.
LINKAGE MAPPING OF ESTS DIFFERENTIALLY EXPRESSED BETWEEN APOMICTIC AND SEXUAL GENOTYPES OF POA PRATENSIS L.


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apomixis, linkage mapping, mode of reproduction, EST mapping

Kentucky bluegrass (Poa pratensis L.) is a hardy, persistent, attractive forage and turf grass adapted to a wide range of soils and climates. The great adaptive capacity of this species is likely associated with its variable ploidy level and versatile mode of reproduction, ranging from obligate apomixis to complete sexuality. Because different plants may show contrasting modes of reproduction, P. pratensis could serve as model species for investigating apomixis and its inheritance.

In the past, data accumulated on P. pratensis suggested that apomixis is controlled by a single genetic locus of undetermined size and structure. This implied that the unreduced egg has a built-in tendency to autonomous parthenogenesis, and that apospory and parthenogenesis are pleiotropic. Recently independent works have, instead, demonstrated that apospory and parthenogenesis are controlled by 2 to 5 genes putatively.

Because of these contrasting data we decided to build a genetic map with the aim of finding marker/s co-segregating with the mode of reproduction. The occurrence of recombination between apospory and parthenogenesis in this species should guarantee the possibility to map the 2 features independently.

Therefore, we developed a population segregating for the mode of reproduction of 124 progeny plants, by crossing an apomictic and a sexual genotype. For all genotypes, the mode of reproduction (aposporic vs. meiotic and parthenogenetic vs. embryogenetic) was investigated through flow cytometry on an average number of 50 seeds in 5 seeds bulks or single seed analysis. Several PCR-based molecular markers (AFLP, SAMPL, SSR, TRAP) were used to build an adequate framework of markers.

Since, a cDNA-AFLP approach allowed us to isolate 178 ESTs differentially expressed between sexual and apomictic genotypes of P. pratensis, our main project aim is to map these ESTs into the PCR-based markers framework to look for ESTs co-segregating with the mode of reproduction (apospory/parthenogenesis). Once one or more ESTs tightly linked to one of the feature of apomixis will be identified, we plan to screen a genomic library (based on cosmodic vectors) to obtain a clone useful for FISH analysis. In this way we could also understand if they are located close to other genes isolated with a different approach and characterized for being differentially expressed (e.g. APOSTART, PpSERK, etc).
CONSTRUCTION AND VALIDATION OF A NEW VITIS VINIFERA MICROARRAY PLATFORM BASED ON COMBIMATRIX TECHNOLOGY

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microarray, grape, gene expression

The Italy-France Grape Genome Project will soon provide the complete sequence of grape genome (about 480 Millions bp). For a better characterization of grape transcriptome, the international community needs a new flexible and comprehensive microarray platform. For this purpose, as members of Grape Genome Project, we have developed a grape chip carrying 24,562 probes based on the last TIGR Vitis vinifera Gene Index (VvGI) release (5.0) and integrated with a non redundant genomic sequences produced by the genome annotation from the Italy-France Grape Genome Project. As new sequences will be annotated by the binational consortium they will be added to the array up. The Grape chip is based on Combimatrix technology, characterized by an high flexibility given by an exclusive in situ oligo (35-40mers) synthesis driven by electrochemistry that allows to reduce drastically fabrication costs. This innovative technology allows the reuse of the microarray for 3-5 times, with a significant reduction of the costs. We have established a reliable protocol from sample labeling to data analysis. 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 data have been obtained up to 4-5 hybridizations with the chip holding 90,000 probes, and up to 6-7 hybridizations with the chip holding 12,000 probes. Reproducibility between arrays was also measured giving similar results. Data obtained with Combimatrix platform were compared with MWG microarray platform and with Northern analyses. Technology reliability was confirmed by a good correlation between the ratios of genes differentially expressed in three developmental stages of grape berry for the three gene expression platforms. Finally platform linearity response and sensitivity were assessed by adding different amounts of RNA spike controls to total RNA samples, giving a resolution of 1 pM.
**VITIS VINIFERA** **CV. PINOT NOIR: DEALING WITH HETEROZYGOUS GENOMES BY MERGING SANGER AND PYROSEQUENCING METHODS**


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Pinot Noir gene prediction, gene annotation, SNP detection, bioinformatics

Given its cultural and economic importance, wine grape is an obvious candidate for the first woody crop to have its genome deciphered. Our research focused on the elite cultivar Pinot Noir with the multiple goals of genome assembly, gene identification and annotation, and identification of a maximum number of polymorphisms. Of special interest to biologists and breeders are polymorphisms in and around the coding regions. Pinot Noir is highly polymorphic with two clearly distinguishable haplotypes revealing several million SNPs and small indels. Based on a conservative estimate, 451,190 new SNPs and 67,580 new indels were revealed during the genome construction. This represents a substantial resource for molecular breeding programs, as well as trait and QTL marker association. Existing software and strategies were not adequate for the assembly of this highly heterozygous genome. We therefore largely focused on developing novel algorithms to address this challenge. A total coverage of seven genome equivalents of libraries of ascending size sequenced by the Sanger method, coupled with systematic highly parallel automated primer walking and 4.2 genome equivalents of 454 Life Science™ sequences, allowed us to create an effective genome sequence. Assembly was then reached by adding sequences of two BAC [0]libraries and a fosmid library which were end-sequenced to assemble large meta-contigs. Contigs were oriented and ordered on appropriate chromosomes by high throughput marker development and genotyping in an F1 cross of Syrah x Pinot Noir. Currently, 59,883 contigs merged into 705 meta-contigs covering 534,5 Mb have been submitted to the GenBank and EBI databases, and are available on the IASMA web site (http://genomics.research.iasma.it) organised in 19 chromosomes.
A HIGH QUALITY DRAFT OF THE GENOME SEQUENCE OF *VITIS VINIFERA* L.

THE FRENCH-ITALIAN PUBLIC CONSORTIUM FOR THE SEQUENCING OF THE GRAPEVINE NUCLEAR GENOME

genomic sequence, genomic assembly, annotation

The grapevine *Vitis vinifera* L. is in economic terms the principal fruit crop in the world. Its haploid genome is estimated to be about 500 Mb, organized in 19 chromosomes. The grapevine is the forth plant whose sequence has been made public, after *A. thaliana*, rice and poplars, and it is the second ligneous and the first fruit plant with a public sequence. Here we present an academic consortium project that completed a 12X shotgun sequence of a quasi-homozygous PN40024 lineage. Sequencing was performed using a Whole Genome Shotgun strategy: all data were generated by paired-end sequencing of cloned inserts from plasmid and fosmid libraries of different insert size, using Sanger technology on ABI3730xl sequencers. Using a 8.4X coverage an intermediary assembly of 498 Mb composed of 3830 scaffolds. Of the 317,364 available *V. vinifera* ESTs 96% were mapped, together with 99.5% of the 38,586 cDNA clones that were produced and sequenced by our Consortium, proving the high genomic coverage of this assembly. The longest scaffold and contig are respectively 12.7 Mb and 557 kb. Half of the assembly is represented by scaffolds longer than 1.9 Mb or by contigs longer than 64 kb. A large majority of these are anchored on linkage groups. Sequence analysis allowed to estimate to less than 7% the residual heterozygosity of the lineage used. Three different approaches revealed that approximately 41% of the grape genome is of repetitive/transposable elements (TE) origin. All classes and superfamilies of TE are represented in the grape genome. The proteome was determined by an annotation strategy reconciling proteins, cDNA alignments and *ab initio* predictions. A large number of duplicated and pseudogenes were found.
miRNA DISCOVERY IN GRAPEVINE BY IN SILICO APPROACHES

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microRNA, grapevine, genomics

The key role of non-coding RNAs in many different biological areas has become clear in the last ten years. microRNAs are a group of non-coding small RNAs (20-22 nt long) which are often conserved between related organisms and which are coded by specific genes called MIR genes. All the transcripts that give rise to the same miRNA are grouped in a single family.

microRNAs are involved in post-transcriptional gene regulation, inducing transcript cleavage or translation inhibition, throughout sequence similarity. Target genes are associated with many different biological processes and possess no significant similarity to their respective miRNA genes, apart from the 21 nucleotide sequence. In plants, target genes are typically involved in stress response and plant development. They are broadly present in the plant kingdom; in recent years many new species have been investigated and many miRNAs have been discovered, mainly through in silico comparative genomic approaches.

Here we present a double in silico approach applied to the complete sequence of the grape (Vitis vinifera L.) genome focused on the discovery of both conserved plant miRNAs and new species-specific miRNAs. Interesting comparative and phylogenetic analyses have been performed that explicit how different miRNA gene families behave during evolution, following gene-expansion or gene-loss, showing in some cases a similar genomic organization, among different species.

Moreover, to further characterize these miRNAs and investigate their putative roles, a detailed target analysis has been performed. As expected targets of related families are highly conserved between species, belonging to the same functional categories. Nonetheless a further characterization of these targets and their corresponding miRNAs can clarify their role and possible involvement in plant growth and fruit development.
IDENTIFICATION OF THE MOLECULAR BASES OF SOMATIC VARIATION IN GRAPEVINE

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Vitis vinifera L., clonal variation, mutation, transposable elements

Grapevine has been vegetatively propagated since its domestication took place 8,000-10,000 years ago. All plants belonging to a certain cultivar are genetically identical to each other and identical to the original plant chosen hundreds or thousands of years ago for some specific merit. Nevertheless, there is natural phenotypic variation within each cultivar. Clonal selection, empirically performed by grape growers, relies on choosing within a cultivar a plant that has peculiar characteristics and that differs from the others belonging to the same cultivar for a specific trait and on its clonal propagation by grafting. The plants obtained from the grafts of the original individual are identified and commercialized as a clone of a certain cultivar. The genetic events, i.e. somatic mutations, that cause the appearance of a plant with distinct characteristics within a set of vegetatively propagated plants are not known. Somatic mutations can occur in the vegetative meristems of buds used to propagate a cultivar and can be caused by nucleotide substitutions and/or indels, transposable element insertions or excisions, epigenetic modifications. These mutations can lead to the appearance of desirable phenotypic variation that is then captured in a new clone. The general objective of our project is to analyze clones belonging to a single variety with high throughput DNA analysis methods to detect differences in DNA sequence that could be ascribed to the sources of variation that were described above (with the exception of epigenetic modifications). While the different grapevine cultivars are well differentiated at the DNA level (with SNPs every 60-70 bp and frequent indels), we expect clones belonging to the same variety to show levels of variation that are several orders of magnitude lower. This analysis is similar in nature to the analysis of somatic mutations leading to cancer in mammalian somatic cells that is currently a very active area of research that is taking advantage of the development of high throughput genome analysis technologies.

We selected 5 pairs of clones belonging to 5 different varieties. Genotyping of each grapevine clone is carried out using 180 different microsatellite loci and approximately 5000 AFLP (amplified fragment length polymorphisms) markers. At present we are also identifying LTR retrotransposons classes that are particularly abundant in grapevine genome in order to use a modified AFLP analysis such as “transposon display” and detect specifically the movement of transposable elements. The observation that in some plants (e.g. maize) active transposable elements of different classes (helitrons, DNA transposons, retrotransposons) are still actively reshaping the genomes and creating variation that could have a functional significance makes this a very interesting area of investigation. Finally 2 pairs of clones will be subject to resequencing of a selected genomic fraction using Sanger sequencing. The sequences produced will be aligned along the assembled WGS sequence of grape and then sequences from the two clones of the same variety
will be compared to detect differences. With all these analyses we hope to determine the frequency and the types of somatic mutations that have occurred over time in these clones. This study will offer an understanding of the molecular bases of the events that underlie most of the phenotypic variation that has been utilized in grape breeding in the last centuries.
GENETIC VARIABILITY AND BIODIVERSITY CONSERVATION OF AN INDIGENOUS GRAPEVINE GERMPLASM COLLECTION: PERSPECTIVE FROM NUCLEAR AND CHLOROPLASTIC SSR VARIATION

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SSRs, chloroplastic markers, germplasm conservation, biodiversity, Vitis

We start a germplasm safeguard program of 19 grapevine varieties considered to be indigenous of North-Eastern Italy. To better estimate how genetic structure of local grapevine germplasm can be used to obtain a conservation perspective, genetic variability at 20 nuclear and three chloroplastic polymorphic microsatellite loci was examined in the autochthonous varieties and in seven European cultivars included as reference. The genetic profiles of all the cultivars were searched for possible parentage relationships and several cases of suspected synonyms were investigated.

A number of synonymous and homonymous varieties were found and three cases of parent/offspring relationships were singled out. The analysis of both nuclear and chloroplastic SSR polymorphisms showed that indigenous varieties present rare alleles and haplotypes absent in the international ones.

Chloroplastic specific haplotypes were pointed out for the first time in this indigenous germplasm and should be considered typical of North-Eastern Italy. The presence of many specific haplotype for the local varieties due to past contribution of wild grapevine to the genepool of cultivated grapevine can be hypothesized. Moreover, most of local cultivars were demonstrated constituting an independent source of genetic variation, and therefore a valuable source of genetic traits for grapevine breeding. Our results can be helpful for the efficient selection of parents in grapevine breeding programs and identification of duplicates and core populations of the local gene pool for the effective conservation and management of the germplasm.
CHARACTERIZATION AND COMPARISON OF STILBENE SYNTHASE SEQUENCES WITHIN EUGANEAN GRAPEVINE CULTIVARS

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Vitis vinifera, stilbene synthase, resveratrol, grape germplasm, resistance gene

Accumulation of phytoalexins and synthesis of PR-proteins are the most frequently observed and the best characterized defense reactions upon fungal infections in grapevine (*V. vinifera* L.).

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 (3,5,4’-trihydroxystilbene), resveratrol glycosides, resveratrol oligomers (viniferins) and pterostilbene.

Resveratrol plays an important role in the resistance of grapevine to colonization by fungi, 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, the last step being catalyzed by stilbene synthase (STS).

Stilbene synthase produces simple stilbenes (*cis*- and *trans*-resveratrol) from *p*-coumaroyl-CoA and three malonil-CoA units, and is encoded by a multigene family of 15-20 members; in grapevine, more than eight resveratrol-producing STS genes have been characterized.

The present work aims at characterizing and comparing the genomic structure of specific stilbene synthase genes in five local grapevine cultivars from the Euganean area, selected within a larger collection on the basis of their genetic diversity and total resveratrol content in ripe berries. Preliminary observations singled out some clones within the local germplasm showing high levels of constitutive resveratrol synthesis, thus suggesting the chance that different genomic variants are present. Furthermore, the potential relationship between induced resveratrol production and the resistance/susceptibility level of the five genotypes following *Plasmopara viticola* artificial inoculation will be discussed.
CONSTRUCTION OF A PHYSICAL MAP OF GRAPEVINE USING A FLUORESCENT FINGERPRINTING STRATEGY

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Vitis vinifera L., fluorescent fingerprinting, physical map

Physical mapping is an active area of genomics research. Fingerprint maps are based upon complete restriction enzyme digestion of large-insert clones representative of the target genome and ultimately comprise a near-contiguous path of clones across the chromosomes of the organism. Such maps are useful tools to enhance positional cloning, support and validate sequence assembly, anchor sequences to linkage maps and provide templates for filling gaps; moreover they are a critical resource for subsequent functional genomic studies, because their overlapping clones provide a redundant and ordered sampling of the genome.

In the frame of the whole-genome sequencing project of grapevine (VIGNA consortium), we built a BAC-based physical map of Vitis vinifera L. using a fingerprint approach. We adopted a High Informative Content Fingerprinting technology developed by Luo et al. (Genomics, 2003, 82:378-389) for cereals and previously adapted to grape in our laboratory. A grape BAC library with a coverage of 16 genome equivalents (70656 clones, average insert size 110 kb) has been fingerprinted. The genotype we chose is PN40024, a Pinot Noir inbred line, obtained after nine cycles of selfing, in order to avoid an expansion of map coverage due to heterozygosity effect (as previously detected in our laboratory in the construction of a Pinot Noir physical map). BAC DNA has been isolated, simultaneously digested with 5 restriction endonucleases, and electrophoresed on a capillary automated sequencer after 4 fluorescent dye labeling. The band pattern produced represents the individual fingerprint of each BAC clone. Raw data have been analysed by the ABI GeneMapper software that performs peak sizing and processed using FPB, an internally developed PERL script, in order to discriminate among true peaks (corresponding to restriction fragments) and background peaks. Finally, a robust contig assembly has been built using the FPC software. The resulting map has a total length that is very close to the true genome size and does not exhibit the scissoring effect emerged in the previously built Pinot Noir physical map; such an effect is due to heterozygosity between the two homologous chromosomes and is reflected into non-overlapping haplotypes assembly.

The aim of our effort is to aid and validate the grape genome sequence assembly and to identify candidate genes of agronomical traits as berry pigments or abiotic stress and pest resistances through integration of physical and genetic maps.
GENE PREDICTIONS AND PRELIMINARY GENE CLASSIFICATION OF PINOT NOIR GENOME

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Pinot Noir genome, gene prediction, gene annotation, bioinformatics

Following the release of Pinot Noir (clone ENTAV 115) genome sequence in public databases the effort was moved from the sequencing to the analysis of the genome, namely the identification and the characterization of genes.

Here we present the methods and the preliminary results of gene prediction and annotation of IASMA genomics pipeline.

Due to the high heterozigosity of the chosen genotype, the current release of the grape genome consists of a large collection of 66,164 contig sequences, for a total of 550 Mbp.

Our gene prediction strategy was based on two kind of "ab initio" programs (GlimmerHMM, FgenesH) and a "knowledge based" one (TwinScan).

In addition more than 320,000 ESTs from GenBank database were used to build 28,856 Tentative Consensus (TC) sequences; then the TCs were aligned on the genome sequence with Sim4 software.

These four sets of data were used to make a "consensus analysis" in order to classify the level of consistency among the different predictions: we took into particular account the gene positions, the exon number and the gene structure. The final set consists of 39,621 gene predictions.

These genes were then clustered together to check redundancy level, searched against retro/transposons and other repetitive elements and annotated with gene ontology terms.
SECONDARY METABOLITE BIOSYNTHESIS IN ELICITED GRAPE CELL SUSPENSIONS

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aroma, elicitors, stilbenes, terpenoids, Vitis vinifera

The aim of this study was to discover genes involved in grape secondary metabolism pathways by stimulating their production in grape cell suspension cultures.

Among numerous elicitors tested, methyl jasmonate (MeJA) induced the production of a wide range of sesquiterpenes in Cabernet Sauvignon cell suspension cultures derived from berries. The efficacy of MeJA in the induction of sesquiterpenes production is strictly dependent upon the density of cells in cell suspension at the moment of elicitor addition. We also found that jasmonic acid (JA) as MeJA activate sesquiterpene synthesis, but when salicylic acid (SA) was added in addition to MeJA, sesquiterpenes were not produced.

Microarray analyses on the induced cultures confirmed the activation of sesquiterpenes biosynthetic pathway by MeJA and JA and the inhibition effect of SA: two terpene synthases on the gene chip were upregulated in the MeJA and JA treated cells.

Microarray data also indicated that genes from tannins and stilbenes pathways are differentially expressed in response to the treatments. Tannins accumulated to higher levels in the MeJA- and JA-treated cell suspension cultures and the expression of pathway key genes was more than 2-fold higher in the MeJA and JA treatments than the control and MeJA+SA cells. Stilbenes accumulated in all three treatments, but the levels correlated mostly with the expression of stilbene synthase (STS2).

The results of this work confirm that an inducible cell culture system is a powerful tool for functional genomics studies as we can associate specific genes from biosynthesis pathways with changes in metabolite production. We are now working towards the functional characterisation of terpene synthases, modifying enzymes and transport proteins associated with volatile production in grapes. We have also identified several transcription factors that have expression patterns matching the induction patterns seen for the three pathways described above. These are also targets of future work as they may help us understand the regulation of these secondary metabolite pathways in grape berries.
CHARACTERIZATION OF BERRY RIPENING AND WITHERING PROCESSES IN *VITIS VINIFERA* CV. CORVINA


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grape, microarray, gene expression, ripening, withering

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

The aim of this work is to identify differentially expressed genes during grape ripening and withering by microarray technique and to correlate the expression results to metabolic and proteomic profiles. Withering 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 grapevine transcriptome of the berry skin of *Vitis vinifera* cv. Corvina (clone 48), sampled in 2006 during three stages of ripening and three of withering, was analysed. For this purpose, a prototype chip based on CombiMatrix technology was developed. This chip allows us to detect the expression levels of about 19,000 TC (*tentative consensus*) of the DFCI database ([http://biocomp.dfci.harvard.edu](http://biocomp.dfci.harvard.edu)). Microarray data will be statistically analyzed to cluster gene expression profile during ripening and withering.

Towards a system biology approach, microarray data will be correlated to metabolics (sugars, acids and polyphenols) and proteomics (DIGE: *D*ifferential *I*n *G*el *E*lectrophoresis) obtained on the same berry samples.
REGULATION OF VACUOLAR pH AND VOLATILE BENZENOID PRODUCTION IN DEVELOPING GRAPEVINE BERRY

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grapevine, benzenoids, vacuolar pH

The grapevine berry has the ability to synthesize many secondary metabolites, some of which are important components towards colour and flavour of both the berry and its product, wine. Phenolics, a class of secondary metabolites, take part in defining colour and some flavour aspects of the berry.

The regulation of colour and the synthesis of volatile benzenoids have been characterized in the Petunia hybrida flower. In this case, colour is defined by the regulation of both anthocyanin synthesis and acidification of the cellular vacuole. Myb, bHLH and WD proteins are involved in the regulation of these two processes. Focusing rather on petunia floral fragrance, a myb protein, ODO1, has been identified as a regulator of production of volatile benzenoids.

The grapevine genomic sequence is an important source to identify and isolate the orthologous genes involved in the regulation of these two berry development processes: vacuolar acidification and benzenoid metabolism. The aim of this work is the characterization of these putative transcription factors involved in vacuolar pH regulation and benzenoid production in Vitis vinifera cv. Corvina (clone 48) berry using the models hypothesized in Petunia hybrida.

References:
ANALYSIS OF GRAPE BERRY PROTEOME DURING RIPENING AND WHITERING

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Vitis Vinifera, proteome, 2-DE, DIGE, plant protein extraction

Grape ripening is a key growth phase for determining the quality of grapes, as it immediately precedes harvesting. Therefore the understanding of molecular mechanisms regulating berry maturation is fundamental to optimize vintage quality. In this work we present the analysis of grape berry (Corvina variety) during ripening and withering processes using a proteomic approach.

Protein extraction from grape berries is a complex process, because of a large amounts of non-protein contaminants, such as polyphenols and pigments, and high sugar levels which interfere with the two-dimensional electrophoresis analysis. We evaluated different procedures based on TCA/acetone and phenol to extract total soluble proteins from various ripening stages (from pre-veraison to withering) of Corvina berries. The TCA-based protocol resulted superior to the phenol method, displaying higher proteins yield and resolution. The optimized extraction protocol was applied to seven ripening stages and the obtained berry proteome maps are being analyzed by DIGE (Differential in Gel Electrophoresis, GE Healthcare) technology.

This comparative analysis will contribute to the knowledge of the physiological role of protein associated to grape berry development and will provide the first dynamic profile of grape berry protein expression during maturation and withering.

The results obtained from this proteomic analysis will be combined with a parallel investigation performed on the same berry samples, using a transcriptomic approach. This combined study may reveal the potential and limits of each methodology approach, in the perspective of applications for quality improvement or characterization of Vitis Vinifera varieties.
TRANSCRIPT PROFILES IN SKIN OF WINE GRAPE BERRIES ARE DIFFERENTLY AFFECTED BY SLOW AND RAPID POSTHARVEST DEHYDRATION RATES

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microarray, gene expression, Vitis vinifera, water stress

The interest of consumers in wines obtained from overriped and/or partially dehydrated grapes has increased in recent years. The main effect of berry dehydration is the concentration of solutes, as sugars and minerals, and the modification of organic acids, aromatic compounds and polyphenol composition with marked effects on organoleptic quality of the berries and the resulting wines. Besides the concentration effects, water loss rate modulates the metabolic pathways. Significant differences have been reported for grape berry composition after dehydration carried out either in uncontrolled conditions (fruttaio) and in controlled room in which the water loss is significantly accelerated. No information concerning transcripts profiles induced by different dehydration rate is available.

A microarray, based on “Grape Genome Oligo Set Version 1.0” containing a total of 14,562 oligos (70 mers), has been used to compare transcript profiles of skins berries (cv Raboso Piave) immediately after harvest (time 0) with those obtained after 10% weight loss reached within 12 or 6 days of dehydration, and 30% weight loss reached within 70 or 30 days. A simple loop experimental design was adopted.

Among the 1,252 targets (~ 8% of all transcripts profiled) differentially expressed, 664 were water loss rate independent while 380 and 208 targets were specifically regulated according to the slow or rapid dehydrate regime, respectively. The composition of these sets will be discussed focusing on polyphenols biosynthesis, cell wall metabolism, stress, and defence responses.
IN INVOLVEMENT OF LEAFY COTYLEDON1-LIKE GENE AND AUXIN IN ECTOPIC EMBRYO INITIATION ON EPIPHYLLOUS LEAVES OF THE EMB-2 HYBRID (HELIANTHUS ANNUUS X H. TUBEROSUS)


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auxin, epiphyll, somatic embryogenesis, LEAFY COTYLEDON1-LIKE gene

The LEAFY COTYLEDON (LEC) genes, LEC1 and LEC1-LIKE (L1L), encode regulatory proteins homolog to the HAP3 subunit of the CCAAT-binding transcription factor. These genes function early in embryogenesis to maintain suspensor cell fate and specify cotyledon identity. Late in the embryogenesis, the LEC genes are required for the initiation and/or maintenance of maturation and the repression of precocious germination. In plants, embryogenesis is not strictly dependent on fertilization because gametophytic and somatic cells can be induced by in vitro systems to undergo embryogenic development. Although the initiation of zygotic and in vitro embryogenesis often arise from different starting tissues and are activated by different signals, it is likely that both processes converge at a very early stage on the same signalling pathway. The clone EMB-2 of the interspecific hybrid Helianthus annuus x H. tuberosus provides an excellent system to study molecular and physiological aspects of somatic embryogenesis. In fact, EMB-2 plants show an unusual pattern of development in that it produces both in vitro and in vivo, epiphyllous embryo- and shoot-like structures. In the present study, we evaluated if H. annuus L1L (HaL1L) and auxin are involved in the initial stages of epiphyllous embryo development. We proved that localized HaL1L ectopic expression precedes the development of epiphyllous structures on EMB-2 leaves. Indeed, HaL1L transcripts were detected in epidermal cells and vascular bundles before ectopic embryos become evident. Histological analysis of EP leaves demonstrated that periclinal division occurring in cells of adaxial epidermis give rise to ectopic structure initiation. Therefore, we propose that HaL1L specifically marks the putative founder cells of ectopic embryos. Given the link between auxin and plant embryogenesis, the endogenous level of free indole-3-acetic acid (IAA) was estimated in epiphyllous (EP) and non-epiphyllous (NEP) leaves of EMB-2 plants as compared to that of a non-epiphyllous genotype (A-2), used as control. The amount of endogenous IAA in EP leaves was higher than in NEP ones, in spite of a quite comparable level in EP and A-2 leaves. In order to verify whether somatic embryo initiation was related to a localized increase of IAA, the hormone spatial distribution was also examined. The pivotal role of auxin as inducing factor of somatic embryogenesis is further supported by the increase of embryo regeneration.
detected in IAA-treated NEP leaves. The monitoring of HaLIL transcription in leaves exposed to auxin treatment allowed us to detect only a slight increase of HaLIL mRNA steady state level as compared to leaves grown on basal medium. However, we cannot exclude that auxin exert its regulatory role on HaLIL either at post-transcriptional and translational level or indirectly acting on other embryogenesis-related transcription factors.

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EFFECTS OF POLYPLOIDIZATION ON GENE EXPRESSION AND DNA METHYLATION IN ALFALFA AND POTATO

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Medicago sativa, microarray, MSAP, polyploidy, Solanum tuberosum

Polyploidy is widespread in plants, to the point that at least 70% of species have experienced polyploidization at some point of their evolution. The modifications that occur as a consequence of chromosome doubling can influence economically important traits. These modifications may be caused by changes in DNA sequence and/or in gene expression. In turn, gene expression modifications may be caused by different mechanisms, and DNA methylation is one of the most important. While the genetic and epigenetic effects of polyploidy are studied in both model and cultivated disomic polyploids, polysomic poliploidy has not received much attention. Technologies are now available for studying these phenomena: microarray technology allows to assess the expression of thousands of genes in a single experiment, and DNA digestion with methylation-sensitive and insensitive endonucleases that recognize the same nucleotide sequence (isoschizomers) allows to assess methylation differences at the level of the whole genome. The objective of our work is to gain insight into the effects of chromosome doubling on gene expression and DNA methylation in two agriculturally important species, alfalfa and potato, polyploids with tetrasomic inheritance. We also aim at the identification of genes that contribute to the phenotypic differences between diploids and tetraploids. This basic knowledge could have practical applications in the breeding of alfalfa, potato and other polysomic polyploids.

In alfalfa, we are studying two diploid (2x) plants of the subspecies *Medicago falcata* and *M. coerulea* that produce 2n eggs and 2n pollen, respectively, their tetraploid (4x) progenies from unilateral and bilateral sexual autopolyploidization, and some triploid progenies. In potato, we are using *Solanum tuberosum* and *S. commersonii* diploid and tetraploid isogenic plants obtained by tissue culture. Polyploidization-induced changes will be investigated by analyzing gene expression for a large part of the genome, by using microarrays of cDNA-derived sequences of *M. truncatula* and potato; and analyzing genomic DNA methylation, by using the Methylated Site Amplified Polymorphism (MSAP) technique. Our preliminary results from the MSAP analysis revealed few methylation changes in the tetraploid genotypes with respect to the diploid ones, both in potato and alfalfa. The implications of this finding are presented from a genetic and evolutionary standpoint.
FORWARD AND REVERSE GENETIC ANALYSIS IN *MEDICAGO TRUNCATULA*

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*insertional mutagenesis, TNT1, Medicago truncatula*

In order to set up a functional genomics tool for forage legumes about 1000 *Medicago truncatula* lines were mutagenized with the tobacco *Tnt1* retroelement.

As a reverse genetic approach about 100 flanking regions from 13 random lines were isolated and sequenced. About 40% of sequences showed similarity with known genes.

Phenotypic screenings were carried out for several traits including plant architecture and forage quality. Three lines, B21, B1 and C43, segregating the mutant phenotype in a ratio compatible with a single locus inheritance, were chosen for further molecular characterization. *Tnt1* flanking regions were isolated and sequence information was used to follow insertion sites segregation.

Here we report the preliminary characterization of the B21 mutant line which shows a strikingly dwarf plant with small leaves and short internodes. Southern analysis showed that parental B21 lines contain at least 5 insertions.

The host flanking region of 4 insertion sites were cloned by Inverse PCR and S-SAP and further characterized. Homologies were found to: HCR4, Peptidyl-prolyl cis-trans isomerase, CHS and the Mth2-81g19 sequenced BAC clone of *Medicago truncatula*.

As far none of the characterized insertions co-segregated with the mutant phenotype, therefore isolation of more flanking regions is in progress.
THE STRAWBERRY FaMYB1 TRANSCRIPTION FACTOR SPECIFICALLY SUPPRESSES CONDENSED TANNINS ACCUMULATION IN LOTUS LEAVES

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tannins, transcription factors, MYB, real-time PCR

The polyphenolic compounds known as condensed tannins (CTs) are plant secondary metabolites obtained from the polymerisation of epicatechin and catechin units and share most of their biosynthetic route with anthocyanins. CTs occur in a wide range of plants where play important role in defence against herbivores and pathogens and have important industrial applications. As food components, they act as antioxidants with beneficial effects for human and animal health. In forage legumes their presence prevents bloating and increases protein assimilation, although too high CT levels decrease palatability. The regulatory machine controlling CT biosynthesis comprises a ternary transcriptional complex consisting of WD40, MYB and bHLH proteins. In order to modulate the tissue-specificity and the level of accumulation of CTs in legumes experiments of transgenesis using both regulatory and structural genes are in progress worldwide. To this purpose, the genus Lotus offers a model system in that it includes species that accumulate different quantity of CTs along with species that accumulate these compounds only in reproductive tissues or in both vegetative and reproductive ones. We have previously shown that as result of transformation with the maize bHLH transcription factor Sn Lotus corniculatus transgenic lines with enhanced or depleted leaf CT levels are produced (Robbins et al. 2003) and that the expression/silencing of Sn tightly correlates with the expression of key structural genes of CTs, such as DFR, ANS, ANR, LAR1 but not LAR2 (Paolocci et al. 2005; 2007). Conversely, the sole expression of Sn is ineffective in inducing CT accumulation when overexpressed in leaves of CT negative model and crop species, suggesting that the expression of MYB partners acting either as positive or negative regulators, rather than those of bHLH or their WD40 interacting proteins, de facto controls the CT synthesis in leaves of a number of species.

Here we report that the overexpression of FaMYB1, a strawberry gene that suppresses anthocyanin and flavonol accumulation in transgenic tobacco (Aharoni et al. 2001), is sufficient to specifically suppress CT accumulation in leaves of S41, the L. corniculatus genotype accumulating the highest amount of these compounds. FaMYB1 reduces the transcript levels of all the structural CT-specific genes, as per real time RT-PCR analysis. The observation of sectorial patterns of CT accumulation in leaves indicates that instable interactions among transcription factors may occur.

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CHARACTERIZATION OF *KNOX* GENES IN *MEDICAGO TRUNCATULA*
SUGGESTED IMPORTANT DIFFERENCES IN THE GENETIC
DETERMINATION OF COMPOUND LEAVES AMONG LEGUME SPECIES

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*homeobox transcription factors, leaf development, Medicago truncatula*

Six *KNOX* genes (*MtKNOXs*) have been identified from *Medicago truncatula* as part of an Italian initiative of post genomics of forage legumes. So far, few *KNOX* genes have been characterized in legumes and little is known about apical meristem formation and vegetative development of these species. Few genetic loci that control legume development have been identified, despite the importance of shoot vegetative growth, morphology and composition in forage and grain legume production and quality. We isolated three class I and three class II *MtKNOX* genes. The predicted amino acid sequences suggested a possible orthology to the Arabidopsis homeodomain proteins STM, KNAT1/BP, KNAT3 and KNAT7 that was confirmed by phylogenetic and conserved structural domain analyses. The STM-like *MtKNOX1* and *MtKNOX6* proteins were shown to retain the capability to interact with the Arabidopsis BELL protein partners of STM and KNAT1/BP. Interestingly, mRNA localization studies carried out on class I *MtKNOX* genes revealed important differences with previously characterised legume *KNOX* genes. *M. truncatula* transcripts were not down-regulated in leaf primordia and early stages of leaf development, features shared with the more distant compound-leaved species *Lycopersicum esculentum*. Our findings suggest that changes in gene expression patterns may contribute to the divergence of shoot and leaf development in close related species.
MAPPING OF KEY HORTICULTURAL TRAITS RELATED TO FRUIT QUALITY, PLANT ARCHITECTURE AND PLANT DEVELOPMENT IN PEPPER (CAPSICUM ANNUUM L.)

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pepper, QTLs, Horticultural traits

Cultivated Capsicum species (Solanaceae) are diploid with a total number of 24 chromosomes (2n=2x=24). The genus Capsicum includes 25 species, 5 of which are cultivated: Capsicum annuum L., C. frutescens, C. chinense, C. pubescens and C. baccatum (Eshbaugh, 1977). Pepper, like most cultivated crops, is subjected to intense breeding efforts by Institutes and private companies around the world. Several traits are usually introduced into elite germplasm from non-commercial, small fruited accessions, thus repeated cycles of recombination/selection (using backcrosses or recurrent selection) are needed to combine favourable alleles controlling climatic adaptation, plant architecture, fruit characteristics and resistance to pathogens. Marker assisted selection (MAS) may be very effective in this process but it may be hampered or slowed down by unfavourable linkages among traits of interest.

We analysed 13 horticultural traits related to fruit quality, plant architecture and plant development, which are under polygenic control, and are used as morphological descriptors of pepper accessions in international germplasm collection. The analysis were performed in three intra-specific pepper populations: (i) 297 F6 RILs from the F1 hybrid “Yolo Wonder” x “Criollo de Morelos 334” (YC); (ii) 114 doubled haploids derived from the F1 hybrid “Perennial” x “Yolo Wonder” (PY); (iii) 101 doubled haploids obtained from the F1 hybrid “H3” x “Vania” (HV), which were previously used for the development of intra-specific genetic maps.

A total of 78, 28 and 33 QTLs were identified in the YC, PY and HV population respectively. QTLs controlling fruit traits were found in chromosomes 1 2, 3, 10 and 11 in all the progenies, while in progeny YC clusters on chromosomes 4 and 12 were also detected. Common major QTLs controlling plant traits clustered on chromosome 2 in all the three populations, while trait-specific and parent specific QTLs on chromosomes 1, 4, 8, 9 and 11 were found, presumably as a consequence of distinct alleles segregating in distinct crosses. Plant traits were found to be under the control of both parental alleles in the YC population, while in the PY and HV populations only two (number of leaves, internode growth time) and one (flowering earliness) traits respectively were controlled by both parental alleles, despite the presence of transgressive individuals for many traits. These results presumably might be explain by the presence of undetected QTLs.

Our results show that 35 out of the 78 QTLs detected control the same traits and are located on the same chromosome in the three mapping populations in study; this makes it possible to
confirm the localisation of genomic regions involved in the control of key horticultural traits such as fruit weight, fruit diameter and fruit shape.
DEVELOPMENT OF TOOLS FOR VALIDATION OF HETEROLOGOUS GENECHIP EXPERIMENTS: POTATO TUBER COLD-INDUCED SWEETENING AS A CASE STUDY

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Affymetrix GeneChip, cold sweetening, Solanum tuberosum, heterologous hybridization

Affymetrix GeneChips have set a standard in terms of reproducibility, sensitivity and other quality parameters even among other oligonucleotide arrays which are usually the most reputed in terms of reliability as compared to other microarray platforms. However, GeneChip availability is limited to a dozen of plant species which of course represent a minimal subset of actively studied organisms.

To help overcome these problems one recently developed approach (NASCarrays Xspecies) exploits signals obtained in preliminary hybridizations with genomic DNA (gDNA) from a species of interest over a “GeneChip-available”, related species and creates an amended CDF file masking signal-devoid probes, thus adapting to some extent the GeneChip to a related species of interest. This approach, however, presents some uncertainties as relies on non-coding nucleic acid hybridization and may reveal technically challenging. We therefore explored a “BLAST-masking” approach, which relies on the wealth of EST sequences to date available for various organisms including plant species. In fact, more than 1.000 ESTs or cDNA sequences are available for 233 plant species and for 40 species there is a sum of 20.000 assemblies + singletons, a value suggesting fair transcriptome coverage. The rationale of BLAST-masking approaches relies in excluding or setting apart from signal calculation poorly matching sequences based on an in silico blasting approach and can either be performed at target level (large scale) or probe level (small scale, for fine-tuning signal calls of a smaller number of genes of interest). In particular, via generation of a list of masked probes within probe sets the small-scale BLAST-masking allows inclusion in GeneChip signal calculation of solely perfectly matching sequences among the two species ruling out mismatch-derived ambiguities. Taking profit of free informatic platforms, (as dCHIP) we have developed specific bioinformatic procedures and tools assisting various aspect of masking procedures. Furthermore, some options as GeneChip background calculation modes as related to heterologous approaches have been investigated by comparing to Real-Time-PCR data.

Our masking approaches have been tested with tomato as GeneChip-available species and potato as organism of interest in the context of potato tuber cold-induced sweetening (CIS), a well-known phenomenon resulting in accumulation of sugars (mainly sucrose, glucose and fructose) at the expenses of starch in cold-stored tubers. Validated heterologous GeneChip data (17 °C control tubers vs tubers incubated 4 days at 4°C) were used to identify cold-responsive gene family members and their transcript accumulation was further monitored with Real-Time PCR over 26 days of cold incubation. In addition to the identification at the sequence level of genes coding for enzymes previously reported to take part to cold sweetening, such as beta-amylases and invertases,
we detect a biphasic behaviour of mRNA accumulation for several sweetening-associated enzymes which escaped previous analyses. Our transcriptional data support a hydrolytic rather than phosphorolytic starch degradation machinery at least within the first month of cold-induced sweetening. Scrutiny of validated GeneChip data further unveils important processes accompanying cold sweetening, and their possible causal contribution to potato CIS is discussed based on circumstantial evidence.
PROGRESSES TOWARDS THE CLONING OF THE TOMATO

PARTHENOCARPIC FRUIT (PAT) GENE

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**COS markers, fruit set, parthenocarpy, positional cloning, tomato**

Our aim is to understand the molecular genetic mechanisms underlying the parthenocarpic fruit (pat) mutation of tomato, a recessive mutation conferring parthenocarpy in tomato (*Solanum lycopersicum* L.) and pleiotropic effects affecting the anthers and the ovules. Expression analysis of genes encoding key enzymes involved in GA biosynthesis showed that in normal tomato ovaries the transcript of *GA20ox1* is in low copy number before anthesis and only pollination and fertilization increase its transcription levels and, thus, GA biosynthesis. In the unpollinated ovaries of the *pat* mutant, this mechanism is de-regulated and *GA20ox1* is constitutively expressed, indicating that a high GA concentration could play a part in the parthenocarpic phenotype. The levels of endogenous GAs measured in the floral organs of the *pat* mutant support such a hypothesis. As genes involved in the control of GA synthesis (*LeT6*, *LeT12* and *LeCUC2*) and response (*SPY*) are also altered in the *pat* ovary, it is suggested that the mutation affects a regulatory gene located upstream of the control of fruit set exerted by GAs.

In addition, we have pursued the positional cloning of the *Pat* gene, by Bulk Segregant Analysis using a set of segregating populations. Former results located the *Pat* locus to the long arm of chromosome 3 between the COS markers T0796 and T1143, previously anchored on the genetic tomato map (EXPEN 2000, www.sgn.cornell.edu). By pursuing the microsynteny between tomato and Arabidopsis, novel PCR-derived COS markers have been developed and mapped inside the target window. T0796 and T1143 display a clear hit with a number of BACs belonging to two plausible unlinked contigs of the tomato HindIII physical map. Hence we have both verified their occurrence and carried out a matching test of the new markers on the two contigs. Moreover, a CAPS marker derived from a BAC end sequence pertaining one of the two contigs was integrated into the target window. The whole data obtained so far allowed us to refine with new anchor-points the genetic region spanning 1.2 cM between T0796 and T1143, and to restrict the *Pat*-containing interval to about 0.2 cM.
THE COMBIMATRIX PLATFORM FOR MICROARRAY ANALYSIS: GENE EXPRESSION IN TOMATO PLANTS INFECTED BY DIFFERENT COMBINATIONS OF CUCUMBER MOSAIC VIRUS AND ITS SATELLITE RNA

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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, alone or in combination with satellite RNA (satRNA) variants, has been undertaken by microarray analysis, at two different time points after infection.

The analysis was performed on the newly developed CombiMatrix platform at the University of Verona, on a tomato chip carrying 20200 specific probes in quadruplicates from assembly of Tentative Consensus of the last Tomato Gene Index (LeGI), release 11.0 (June 21, 2006).

The CombiMatrix CustomArray™ technology is characterized by an exclusive in situ oligo (up to 40 mers) synthesis driven by electrochemistry and by the reusability of the same microarray chip, all factors that confer high flexibility to the system and reduce remarkably the costs of microarray analysis.

Tomato cv. UC82 plants were infected with four different CMV strains: CMV-Fny and three more strains, in which CMV-Fny is associated with three satRNAs (benign variant: CMV-Fny/Tfn-satRNA; stunting variant: CMV-Fny/TTS-satRNA; necrogenic variant: CMV-Fny/77-satRNA). Mock-inoculated plants were used as the negative controls. Gene expression was examined at 2 and 9 days post-inoculation, to analyse early transcriptional changes associated with the development of different symptoms in both locally and systemically infected leaf tissues.

Hybridisations were carried out with samples deriving from three independent biological replicates. Differentially expressed genes were selected and analysed using the multi experiment Significance Analysis of Microarray test, and gene clustering was performed using Genesis software. This is the first time that the CombiMatrix platform for microarray analysis has been applied to the study of tomato-virus interactions.
GENETIC RELATIONSHIPS AMONG **NICOTIANA** SPECIES OF SECTION TOMENTOSAE

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**Nicotiana, Tomentosae, ISSR**

The genus *Nicotiana* is a member of the family Solanaceae and contains about 76 species divided in 14 sections and 3 subgenera (*Rustica, Tabacum* and *Petunioides*). According to the authoritative monograph of *Nicotiana* by Goodspeed, 5 *Nicotiana* species were placed in the section Tomentosae (subgenus *Tabacum*): *N. tomentosiformis*, *N. tomentosa*, *N. otophora*, *N. setchellii* and *N. glutinosa*, all having a chromosome number 2n=2x=24. Recent phylogenetic studies suggest that *N. tabacum* (2n=48, section Genuinae) and *N. kawakamii*, a recently discovered species, should be included in section Tomentosae, while *N. glutinosa* should be included in section Undulatae. Moreover further studies, within section Tomentosae, report that the karyotype of *N. kawakamii* is more similar to that of *N. otophora* than that of *N. tomentosiformis*; in spite of this, highly repeated DNA sequences present in *N. kawakamii* have not been found in *N. otophora*.

The aim of this work was to assess the genetic relationships among *Nicotiana* species of section Tomentosae by morphological investigations and ISSR (Inter Simple Sequences Repeats) molecular analysis. *Nicotiana* species of section Tomentosae and some species belonging to the other sections of the genus were grown in pots in greenhouse. During the vegetative cycle morphobiometrical data were collected, in particular as regards the flower parameters. Genomic DNA was extracted and amplified utilizing 8 different 3’ and 5’ anchored ISSR primers, previously selected on the basis of the clarity and reproducibility of bands. All the assayed primers revealed polymorphic patterns. A total of 200 bands was scored, in the size ranging from 200 to 2000 bp. Genetic similarities were calculated according to Jaccard’s Similarity Index and used to construct a dendrogram based on UPGMA. The cluster analysis grouped together *N. kawakamii*, *N. tomentosiformis*, *N. tomentosa*, and *N. otophora*. *N. kawakamii* resulted more related to *N. tomentosiformis* than *N. otophora*. *N. tabacum* and *N. sylvestris*, a maternal progenitor of tobacco, clustered together and resulted the most related species to Tomentosae group. *N. glutinosa* fell outside these two clusters.
INSIGHTS INTO MELON/FUSARIUM OXYSPORUM F. SP. MELONIS INTERACTION THROUGH LARGE-SCALE TRANSCRIPTION ANALYSIS


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transcriptomic, plant disease, soilborne pathogen, Fusarium wilt

_Fusarium oxysporum_ f. _sp. melonis_ (FOM) is the causal agent of a severe vascular wilt disease of muskmelon (Cucumis melo) worldwide. Four races are presently known (0, 1, 2, and 1,2), one of which, race 1,2, is able to overcome the resistance of commonly cultivated resistant varieties. No genes have been identified in muskmelon that confer high levels of resistance to race 1,2. In this context, it would be very important to understand the molecular basis of resistance and susceptibility in melon, and of virulence in the pathogen.

A transcriptomic approach has been undertaken by cDNA-AFLP on melon plants cv. Charentais-Fom2 infected with race 1 (avirulent) and race 1,2 (virulent), at 2, 4, 8 and 21 days after inoculation. RNA from fungal colonies of the two races was also included in the analysis, to identify possible fungal transcripts expressed in plant during infection. A total of 1,376 differentially expressed bands were detected by running 128 primer combinations. All these bands have been clustered in expression profiles as follows: a) genes modulated in the incompatible interaction or b) in the compatible interaction only; c) genes modulated in both interactions with different profiles d) genes expressed in plant, but showing a band of similar size also in the fungal samples, which might be of fungal origin. All cDNA fragments have been eluted from the gels and will be sequenced for homology search in databases. Few differences in gene expression have been detected between virulent and avirulent race grown in culture, which will be the basis for race characterization.
CLONING OF KENAF (HIBISCUS CANNABINUS L.) MAJOR LIGNIN AND CELLULOSE BIOSYNTHESIS GENE SEQUENCES AND THEIR EXPRESSION ANALYSIS DURING PLANT DEVELOPMENT

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kenaf, lignin, cellulose, gene cloning, qPRC

Lignin and cellulose are the high abundant biopolymers in plant cell wall. In the recent years, the demand of vegetable fibers has increased steadily, due to their good mechanical and biodegradability proprieties and due to the increase of biocomposite material production. However, biocomposite industry requires vegetable fibers with features that are closely at the industrial requirements. Since the rheological properties of biocomposite materials deal with the total content and quality of lignin and cellulose-composing fibers, biosynthesis knowledge of those two important biopolymers is an essential phase in biotechnologically-oriented plant breeding.

Kenaf (Hibiscus cannabinus L.) is an important herbaceous plant cultivated mainly as source of the two above-mentioned fibers. In particular, lignin fibers are mainly present in the stem cortical external part (bark); the internal part (core) furnishes light and absorbent wood due to its high content of cellulose.

The final goal of our research group is to modify quantity and quality of kenaf fibers for specific industrial uses. This genetic approach, however, needs the basic scientific knowledge of the time-course expression of the major genes involved in lignin and cellulose biosynthesis pathways and the related final compound biosynthesis quantity during the plant growth. We focused our research on four lignin biosynthesis genes (cad, cinnamyl alcohol dehydrogenase; ccr, cinnamoyl-CoA oxidoreductase; c4h, cinnamate 4-hydroxylase; 4-cl, 4-coumarate:CoA ligase) and on one of the cellulose synthase gene family (CeSA1).

As reported previously (Ruotolo et al., Proc. SIGA Congress, B.48, 2005) a bioinformatics approach was applied to design primers on highly conserved coding sequences. Partial sequences of the above mentioned genes were cloned and nucleotide sequences obtained from genomic DNA and cDNAs. Comparison sequences analysis, based on nucleotide or amino acid residues, for each single gene has shown high identity percentage to other plant genes, mainly with other Malvaceae species like cotton (Gossypium spp.). Following a similar approach, a sequence of a kenaf actin gene was isolated and cloned, in order to have a reference for the further gene expression analysis.

Kenaf cv. Dowling plants were grown under environmental controlled conditions in a growth chamber. Analyses of c4h, cad and CeSA1 gene expression were carried out on mRNAs extracted from bark and core from 30, 60 and 90 days after sowing. mRNA from the kenaf actin gene was used as internal control. qPCR analysis has shown the variation of each gene expression both during plant growth and between core and bark.
REGULATION AND METABOLISM OF DICAFFEOLQUINIC ACID IN ARTICHOKE

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Artichoke is a promising source of bio-pharmaceuticals and its leaf extracts have been used in herbal medicine as hepatoprotectant and choleretic. The major pharmaceutical compounds of this plant belong to the category of polyphenols, with cynarin (dicafeoylquinic acid) and chlorogenic acid (caffeoylquinic acid) as the most abundant molecules.

The aim of our study is to acquire new knowledge in the metabolism of dicafeoylquinic acids in artichoke, by studying the regulation of these compounds in relation to abiotic stresses, and their biosynthetic pathway.

It is known that the biosynthesis of phenylpropanoids is developmentally activated in specific tissue and cell types and also in response to biotic and abiotic stimuli, such as wounding, pathogen infection and ultraviolet radiation. In response to inevitable exposure to damaging UV radiation, plants have evolved UV-induced mechanism of protection and repair, such as accumulation of UV absorbing pigments like flavonoids.

To test regulation of caffeoylquinic compounds by abiotic stress, we treated foliar disks of artichoke shortly with UV, and compared them to untreated disks. The HPLC profile after a range of recovery times showed that the short UV treatment induces synthesis of caffeoylquinic compounds, a maximum after 24h UV treatment.

Most plant phenolics compounds are a product of phenylpropanoid pathway which catalyzes the conversion of phenylalanine to a myriad of phenolic secondary metabolites. The metabolism of dicafeoylquinic acid in plant is still unknown. The biosynthesis of the precursor, chlorogenic acid, occurs through the activity of transferases, like HCT (hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyltransferase) and HQT (hydroxycinnamoyl-CoA quinate: hydroxycinnamoyltransferase) we have already isolated in artichoke, and which transfer a caffeoyl unit to quinic acid. Our hypothesis is that a related caffeoyl transferase could be the missing link in the pathway to cynarin.

We detected an enzymatic activity, using plant enzymatic extracts, that produces a compound with the same absorbance spectrum and similar retention time of cynarin.

Future effort will go in the direction of confirming the identity of this compound with Lc/-Ms analysis, correlating UV stress and enzymatic activity and isolating the enzyme responsible for this chemical reaction.
TOWARDS A PROTEOMIC APPROACH IN ARTICHOKE

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artichoke, Proteomics, Low-abundant proteins, RuBisCO, PEG

The globe artichoke (Cynara cardunculus var. scolymus L., Compositeae) has long been used for culinary purposes, but also has a number of known therapeutic effects. Phenylpropanoids (PPs) and sesquiterpene lactones (SLs) are the major components of its health promoting effects. PPs are cellular protectants against oxidative damage, hepatoprotective and choleretic agents. SLs display immuno-modulatory effects on macrophages/monocytes, regulates virus-induced chronic inflammation, and prevent the invasion of leukocyte cancer cells. At present, little research effort has been devoted to improve our understanding of the artichoke's secondary metabolism and functional genomics. Here we report on the adaptation of a proteomic approach to artichoke in order to study the above mentioned pathways.

Two protein extraction methods from young leaves, broadly used for plants (TCA acetone and phenol based), were applied and a 2-Dimensional electrophoresis (2-DE) protocol was set up according to O'Farrell’s (1975, J. Biol. Chem. 250: 4007–4021), with some modifications. All the resulting 2-DE maps were characterized by the presence of high-abundants proteins, such as ribulose-1,5-biphosphate carboxylase-oxygenase (RuBisCO), which tend to hide the low-abundants ones and affect gel resolution.

To circumvent this problem, a differential PEG precipitation approach was applied, which made it possible to separate the PEG fractions containing RuBisCO from the others. This increased the resolution as well as the total number of detectable proteins in artichoke 2-DE gels.

Protein extraction was carried out using Magnesium/Nonidet-P40 (Mg/NP40) buffer. Total extract was fractionated with PEG into four fractions that were further precipitated with TCA/acetone, in order to remove interfering compounds. Proteomic maps were produced starting from both total extract and PEG fractions. All the maps were replicated three times.

To validate our approach, 2-DE gels obtained from total extract and PEG fractions were compared with Image Master 2D Platinum software (GE Healthcare) and finally a synthetic foliar artichoke proteomic map was built up. As expected, the resulting 2-DE maps generated with PEG fractioning showed more clear patterns, a reduced representation of RuBisCO and new protein spots, otherwise undetectable with conventional extraction methods.

To our knowledge this is the first proteomic approach applied to artichoke bearing proteomic maps. Future studies will be addressed to investigate the artichoke proteome under stress conditions (UV-C and osmotic treatments) so that to identify key enzymes/regulators differentially expressed in the phenylpropanoids and sesquiterpene lactones metabolisms.
DIFFERENCES BETWEEN VIRUS FREE AND CONTROL ARTICHOKE PLANTS OF THE SAME Clone DETECTED BY ISSR AND AFLP MOLECULAR MARKERS

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Cynara, ArLV, hitchhiking, molecular markers

The globe artichoke (Cynara cardunculus var. scolymus) is a field-grown vegetable crop widely used in the Mediterranean region. Italy is the world leader in globe artichoke production, with about 50,000 ha cultivated annually. Globe artichokes are generally propagated vegetatively by offshoots, stumps or dried shoots harvested from commercial fields at the end of the production cycle; however, the potential for the spread of pest (nematodes, fungi and viruses) using the current propagation technique is very high leading to significant economic losses. Sanitary status of artichoke crop is seriously compromised by viral infections, which reduce yield and quality of production. For this reason, European regulations foresee the absence of the most dangerous pathogens, including Artichoke Latent Virus (ArLV), from the commercial propagated material. Some virus-free plants of global artichoke clone ‘C3’, obtained from meristem tip culture, have been evaluated together with their virus-infected plants (control) at the ARSIAL’s experimental farm in Tarquinia (Viterbo, Italy). The RNA extracted, at the same phenological period, from 5 virus-free plants and from the correspondent 5 control ‘C3’ plants, have been used to obtain C-DNA by RT-PCR, and perform differential analysis utilizing both ISSRs and AFLPs molecular markers. Even if the plants within groups are clones, so genetically uniform, different patterns were found within plants of the same clone. This could be probably due to a general high heterozygosity and phenotypic plasticity existing in the global artichoke. Despite that, comparing the two thesis (i.e. virus-free versus control plants) the results underline a general higher number of amplification bands in the control plants than from the virus-free plants; indicating a higher number of transcribed genes in the virus-infected plant than in the virus-free ones. Moreover, some differential band between virus-free and control plants have been found.
GENETIC VARIABILITY ANALYSIS BY MOLECULAR MARKERS IN “PIETRELicina ARTICHoke” POPULATIONS

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artichoke, ISSR, SSR

Pietrelcina artichoke, besides the well known alimentary and therapeutic properties of such a vegetable, is characterized by a very special taste for its fragrance, softness and delicacy. To valorize such a product which is very appreciated and much in demand on the market, this research concerned the evaluation of genetic variability by molecular markers in “Pietrelcina Artichoke” populations. Intra- and inter- populations genetic variability was evaluated on 10 genotypes chosen at random in three farms of Pietrelcina area. The DNA extracted from each of the thirty individuals and from artichokes belonging to the main variety groups, was examined utilizing SSR and ISSR molecular markers.

SSR analysis was performed with 18 primers chosen from recent literature. No polymorphism among Pietrelcina artichoke populations as well as Romanesco type and Pietrelcina artichoke populations was revealed.

ISSR analysis was performed with 8 primers, previously selected on the basis of clarity and reproducibility of bands. They generated from 6 to 17 amplification products having a size ranging from 400 to 1700 bp. A total of 94 bands were scored. The results revealed a very low polymorphism both among the individuals of the same population and among the three populations of Pietrelcina. Genetic similarities were calculated according to Jaccard’s Similarity Index and used to construct a dendrogram based on UPGMA. The cluster analysis grouped the populations of Pietrelcina artichoke together with Romanesco type.

The analysis carried out by both type of markers didn’t allow to characterize a particular profile for “Pietrelcina Artichoke” and underlined, according to capitulum morphology, their belonging to Romanesco type.

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MULTIGENE FAMILY DOMAIN POLYMORPHISM DISPLAY IS USEFUL FOR DEVELOPING FUNCTIONAL LINKAGE MAPS

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MFDP markers, linkage maps, apomixis, Hypericum perforatum L.

Since the introduction of the amplified fragment length polymorphism markers, a number of AFLP-derived protocols has been performed with different purposes. M-AFLP, SAMPL, S-SAP, and TRAP are only a few examples among the reliable and informative marker systems so far used for genome fingerprinting and gene mapping in plants. Multigene family domain polymorphism (MFDP) is another attractive AFLP-derived molecular marker system we ideated and exploited with the aim of displaying markers related to expressed genomic regions and constructing functional linkage maps for sexually and apomictically reproducing plants of Hypericum perforatum L.

St. John’s wort (Hypericum perforatum L.) is an attractive model system for the study of apomixis, mainly because it is characterized by a relatively small genome size and a versatile mode of reproduction, ranging from complete sexuality to nearly obligate apomixis. Its wild populations are composed mainly of tetraploids (2n=4x=32), although diploid (2n=2x=16) and hexaploid (2n=6x=48) chromosome numbers have also been reported. It is known that diploid genotypes are sexual whereas polyploids reproduce by pseudogamous facultative apomixis.

Molecular markers and linkage maps are basic investigative tools which have not been extensively used to analyze the genetic control of apospory, parthenogenesis and apomixis within this species. The fine mapping of the chromosomal regions that control the expression of apomixis is a major requirement for the isolation or validation of candidate genes for apomixis.

The detection of multigene family domain polymorphism markers relies on the amplification of coding sequences that belong to different multigene family members shared among plant organisms. Multi-locus PCR-based genomic fingerprints can be generated by using heterologous primer sets designed on the most conserved gene domain stretches in combination with standard restriction-site related AFLP primers. With this respect, eight conserved domains were analyzed with a minimum of one to four different primer combinations.

As plant materials, two diploid sexual plants isolated by F. MATZK (IPK, Gatersleben, Germany) within German ecotypes and chosen for antagonist morphological traits were crossed and the hybrid segregating population was analyzed by means of AFLP and MFDP markers. Genetic and bioinformatic data of the first linkage maps for diploid Hypericum perforatum L. based on multilocus molecular markers of known sequence and putative function are reported and major results discussed. Tetraploidized sexual plants were recently crossed with tetraploid apomictic...
plants and the F$_1$ population is currently assayed for segregation of apomixis components and molecular markers.
APOSPORY IN HYPERICUM PERFORATUM: FROM A CYTOHISTOLOGICAL ANALYSIS OF SPOROGENESIS AND GAMETOGENESIS TO THE TRANSCRIPTOME

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megasporogenesis, megagametogenesis, apospory, transcriptome

In plants, sexual reproduction is composed by a coordinated series of events culminating in the meeting of male and female gametes during fertilization. It is well documented how both male and female gametophytes originate from specific cells through megasporogenesis and megagametogenesis. Both processes are under complex genetic control and influenced by numerous factors which are not well understood.

In contrast to sex, a number of plant taxa can produce seed via an asexual phenomenon known as apomixis. In one form of apomixis called apospory, the development of one or even more functional embryo sacs occurs from somatic cells of the nucellar tissue. These cells are known as the aposporous initial cells. Within these aposporously-derived embryo sacs, an unreduced egg cell develops parthenogenetically into an embryo, and endosperm can be formed either with (i.e. pseudogamous) or without (i.e. autonomous) fertilization. Recent studies suggest the adoption of Hypericum perforatum as a model species to study this type of apomixis, as it is characterized by a relatively small genome size and a versatile mode of reproduction, ranging from complete sexuality to nearly obligate apomixis. Its wild populations are composed mainly of tetraploids (2n=4x=32), although diploid (2n=2x=16) and hexaploid (2n=6x=48) chromosome numbers have also been reported. It is known that diploid genotypes are sexual whereas polyploids reproduce by pseudogamous facultative (i.e. a single individual produces both sexual and apomictic seed) apomixis.

With the aim of studying the functional genetic basis of the aposporic developmental pathway, we have initiated an ovule-specific analysis of the Hypericum perforatum transcriptome. This will be done by using massively parallel sequencing methods (e.g. 454 technology) to sequence the complete transcriptome of microdissected sexual and apomictic ovules. We are thus performing deep cytological analyses in order to define the developmental window and location of aposporous initial cell development. This information will provide us the information required to guide our tissue microdissection efforts for subsequent sequencing analyses. With this respect, tissues from plants of known sexual and aposporic behaviour were harvested at different developmental stages, and both female sporogenesis and gametogenesis were investigated by means of whole mount microscope analysis.
LOW TEMPERATURE EFFECT ON HOUSEKEEPING AND SUCROSE SYNTHASE GENES EXPRESSION IN SUGAR BEET

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sugar beet, real-time PCR, internal control, sucrose, normalization

An integrated approach using advanced bioinformatics tools and targeted gene expression analysis was carried out to evaluate the potential use of seven “housekeeping” genes, commonly employed as internal controls in real-time PCR analysis. Adequate testing of reference gene consistency, is always necessary to validate data from any new experimental conditions, since a unique “housekeeping” suitable for every species, organ, developmental stage and treatment does not exist. Thus, the expression stability of glyceraldehyde-3-phosphate dehydrogenase, elongation factor 1_, actin 11, beta-6 tubulin, polyubiquitin 10, 18S rRNA and 5S rRNA genes were evaluated after exposure to low temperatures and in different organs of \textit{Beta vulgaris} ssp. \textit{vulgaris} plantlets. The cDNA sequences were derived from GenBank (NCBI) and TIGR-BvGI (\textit{Beta Vulgaris Gene Index}), a platform providing high-fidelity tentative consensus (TC), obtained by a reliable and stringent ESTs analysis.

Transcripts levels were quantified in roots, leaves and cotyledons of 4 weeks-old, hydroponically grown sugar beet plantlets at control temperature (23°C), and upon three hours of low temperature exposure (0°C, -2°C and -4°C). Based on the “comparative Ct method” and the index of stability (M), calculated by GeNorm software, results indicate that tubulin, rRNA and elongation factor1_ were the most stable reference genes respectively for inter-tissues comparative analysis, and for leaves, cotyledons and roots. Depending on the organ under study, the geometric mean of the two most invariant genes was used in order to compensate possible small “housekeeping” gene expression fluctuations, and normalize the data of expression of the target genes. This work underlines the importance of “dry/wet” analysis, especially for those species with little molecular information available, as sugar beet, and confirms the necessity to normalize expression data with appropriate internal controls to guarantee the accuracy of the results.

Sugar beet sucrose synthases 1 and 2 (SBSS1 and SBSS2) genes expression was investigated by relative quantification; the results indicate organ-specific variability in expression (especially high in roots and cotyledons), and stress-modulated transcription levels in some conditions. Roots of young sugar beet plants expressed SBSS1 and SBSS2 at much higher levels than the leaves (12- and 790-fold higher, respectively). In the leaves, induction of transcription of both synthases was observed especially upon exposure to 0°C temperature, at levels of about 15 times compared to control plants. These data seem to support the hypothesis that SBSS genes are cold-responsive genes (COR).
EXPLORING THE COLD TRANSCRIPTOME OF BETA VULGARIS

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sugarbeet, cold stress, cDNA AFLP

Sugarbeet (Beta vulgaris ssp. vulgaris) is generally sowed in spring, but a ever more diffused practice is early sowing. Early spring sowing has several advantages for root productivity, however, a major drawback is the risk of exposing young sugar beet plantlets (at the cotyledon stage or shortly later) to low or even freezing temperatures during the night. Molecular mechanisms underlying the sugar beet response to low temperatures are still unknown, and only few information about cold-responsive genes in the genus Beta are available; therefore, the necessity to investigate this aspect of sugarbeet molecular biology.

Low temperature treatments were applied to Beta vulgaris ssp. vulgaris (cv Bianca) plantlets, at the first leaf stage; hydroponic culture were set up as experimental system to simulate the temperature stress. Plantlets grown for about three weeks at 22 °C-17 °C (day-night temperatures), were exposed to two different cold treatment: 0°C and -2°C for 3 hrs. At these temperatures the plant survival was 100% for controls and 0°C treatment, and 70% for plants exposed to -2°C. In some experiments the plants were also exposed to 5 °C for 12 hrs before cold stress, as a hardening treatment. Transcript profiling of the sugar beet response to low temperatures was carried out by cDNA-AFLP. Two restriction enzyme combinations were used, respectively AseI/TaqI and BstYI/MseI, in order to obtain a more complete coverage of the whole transcriptome, as predicted by their restriction site distibutions in the Arabidopsis transcriptome. Streptavidin-coated paramagnetic particles and biotinylated oligo-dT probes were used for mRNA enrichment of total RNA preparations, for double stranded cDNA synthesis and double digestion, allowing the production of nearly one restriction fragment per transcript.

Selective amplifications with 38 different combinations of Ase-NN/Taq-NN primers produced 48 modulated TDFs and 12 transcript fragments, putatively derived from constitutively expressed genes. Some of the putatively differential TDFs were also modulated in a tissue-specific and treatment-specific mode. All the modulated TDFs were eluted, checked on agarose gel after selective amplification and directly sequenced. Single run direct sequencing of the rescued fragments produced only few sequences that were analyzed with the BLAST algorithm, allowing the identification of two sequences with a high degree of similarity respectively with a J-domain protein (a molecular chaperone), and an ABA- inducible protein. The preliminarily cDNA AFLP analysis of Beta vulgaris cold transcriptome with BstYI-N/Mse-NN, -NNN primer combinations produced on average more TDFs than Ase/Taq combinations, probably due to the intrinsic variability of the BstYI restriction site.

All the TDFs produced by the Ase/Taq and BstY/Mse cDNA-AFLP analysis were cloned and sequenced for BLAST analysis; RT-PCR was carried out to confirm their low temperature or hardening treatment modulation.
SEQUSCING OF KALANCHOE XHOUGHTONII KNOTTED-LIKE GENES AND ESTABLISHMENT OF PHYLOGENETIC RELATIONSHIPS

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Kalanchoe xhoughtonii, vegetative vivipary, knox genes, phylogeny

Vegetative vivipary leads to formation of novel complete plantlets on mature organs, arising without the sexual process. Among Crassulaceae, vivipary has been reported in many Kalanchoe spp., on leaves, stems and flower stalks. The plantlets formation on leaf margin is species-characteristic, ranging to unmetamorphosed buds in K. laxiflora Baker to somatic embryo in K. pinnata Persoon (Batygina, 2005). 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. They fall down from the parent plant and start to grow, having no dormancy.

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. There are several reports suggesting that this class of homeotic genes could be involved in vivipary. In the dominant barley Hooded mutation localized over-expression of bkn3 leads to de novo formation of a meristem, with consequent flower development on the lemma in place of the awn (Müller et al., 1995). Moreover over-expression of bkn3 in transgenic tobacco reveals iterated occurrence of epiphyllous shoots on leaves of progenitor epiphylls. bkn3 seems trigger ectopic expression of resident tokn1 and tokn2 within the epiphyllous meristem (Müller et al., 2006). Ectopic meristems development on leaf also occurs when class 1 knox genes are constitutively expressed in several simple-leafed species like maize, rice, tobacco and Arabidopsis (Lincoln et al., 1994). Conversely class 2 knox genes are expressed in several tissue and their functions are not clear yet.

In an attempt 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. In order 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). Preliminary results show that predicted protein of KxhKN5 gene cluster with KNAT1 subgroup, together with bkn3; this subgroup is well distinguished from STM group. Within class 2 genes, KxhKN1, KxhKN3, KxhKN4, cluster in a group, whereas KxhKN2 is more distant from them.

LARGE-SCALE PROTEIN CHARACTERIZATION OF STRAWBERRY FRUIT


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strawberry, ripening and fruit quality, proteomics

Strawberry is one of the most popular fruit and it is worldwide appreciated for its unique flavour as well as for the nutritional qualities. During the ripening process, changes in fruit firmness, pigmentation and sugar content contribute to confer berry characteristics remarkable on the market. Here we report the first attempt to elucidate at proteomic level the complex physiological process of strawberry fruit ripening using a combination of different methodologies.

For our studies we used a strawberry cultivar (Queen Elisa) characterised by interesting quality traits. Protein extracts from fruits at three different ripening stages (white, turning and red) were analysed by multiple proteomic approaches. About 300 hundred proteic components were resolved on two-dimensional electrophoresis (2-DE) gels (pH range 3-10L). As a first step protein spots having overlapping coordinates among the compared stages were analysed by nLC-ESI-IT-MS/MS. Peptide MS/MS data were then searched against publicly available protein and EST databases, in order to create an annotated proteomic map of strawberry fruit. We are currently using this map as a reference to investigate differences in protein expression during ripening through statistical analysis (by “Decyder” software, GE Healthcare).

Moreover, to evaluate limits and potential of different technological approaches for the study of the strawberry fruit proteome, we adopt in parallel a second methodology based on shotgun peptide analyses. The complex protein mixtures were digested by a protease to produce a large collection of peptides, which were then subjected to two-dimensional liquid chromatography coupled on-line to the tandem mass spectrometry. In order to complete the proteomic analysis on the elite genotype Queen Elisa and to correlate fruit protein expression with quality traits of this cultivar, we have also identified a number of proteins differentially expressed between this cultivar and its parentals (Miss and USB35) by 2-DE DIGE system (GE Healthcare). Protein identification of selected differential spots in under investigation by mass spectrometry.

Despite of the lack of strawberry species-specific sequences in protein and nucleotide databases, a significant number of proteins were identified. This was accomplished by the combination of gel-based and gel-free approaches for comparative proteomic analysis, providing the first characterization of the strawberry fruit proteome and the description of its variation during ripening.

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COMPARATIVE ANALYSIS OF EXPRESSED TRANSCRIPTS TO INVESTIGATE ON TISSUE-SPECIFIC GENES


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Citrus sinensis (L) Osbeck, flesh, array, differential gene expression, anthocyanins

We collected 94.127 Citrus sinensis (L. Osbeck) EST sequences from different cDNA libraries in a secondary EST database. The collection comprises also ESTs from the Citrus sinensis flesh suppression subtractive hybridization (SSH) cDNA library we constructed. These sequences are from the Cadenera (common orange) and the Moro nucellare 58-8D-I (blood orange) cultivars.

All the sequences collected are used to feed a clustering/assembling procedure in order to assemble unique transcripts [tentative consensus sequences (TCs) + singletons] from orange mRNA sequences. Furthermore, data from Gene Expression Analysis using oligonucleotide arrays are considered too. Two chip array have been used: a cDNA chip array whose probe-sets were designed using the EST sequences from the Citrus sinensis flesh SSH cDNA library and the Affymetrix GeneChip Citrus Array. Samples of mRNA extracted from the flesh tissue were hybridised onto both the arrays.

In order to investigate on flesh-specific genes, we compared the results we obtained from the different methodologies.

Our attention was focused on the screening and analysis of transcript sequences which resulted specifically and uniquely expressed in the flesh tissue. We identified 71 TCs generated from multiple sequence alignments of at least 2 ESTs. 16 out of the 71 TCs are assembled from a cluster of ESTs which includes sequences from the Citrus sinensis flesh suppression subtractive hybridization (SSH) cDNA library. 13 out of 16 are the TCs which are solely assembled from ESTs belonging to the SSH cDNA library.

Considering these 16 transcripts, 11 are spotted on the cDNA chip array and 9 of them are up-regulated in blood oranges; 7 have a match with the Affymetrix chip, 4 out of 7 are spotted also in the cDNA chip array and 2 of them are upregulated also through Real time PCR. Our preliminary results show that the flesh specific genes of C. sinensis encode for structural enzymes of the anthocyanins pathway, for genes involved in the flavour mechanism, in the organization of the cell wall and in senescence or for transcription factors. The perspective of the work is to exploit the data at the light of co-expressed genes from various analytical methods and to set up an automated approach to compare different results from the different approaches.
MOLECULAR AND BIOCHEMICAL CHARACTERISATION OF LIPOXYGENASE GENE FAMILY IN HAZELNUT (CORYLUS AVELLANA)*

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gene family, lipoxygenase, Corylus avellana

Lipoxygenases (LOX, E.C. 1.13.11.12) are ubiquitous non-heme iron-containing dioxygenases that catalyse the hydroperoxidation of polyunsaturated fatty acids with a cis,cis,1,4-pentadiene structure.

In plants, the most common substrates are linoleic (C18:2) and linolenic (C18:3) acid. Although many data are available on the structure and the biochemical activity of lipoxygenases, their biological role is still under investigation. It has been ascertained that lipoxygenases are involved in several important physiological processes such as germination, development, senescence and in plant responses to biotic and abiotic stresses.

In a previous work we described the biochemical and molecular characterization of a new lox gene (CaLOX1) early expressed during hazelnut seed (Corylus avellana) development.

Here we report the isolation and molecular characterization of a new member of the lox gene family present in the hazelnut genome. This gene, named CaLOX2, showed high identity towards CaLOX1 and is strictly related to other LOXs previously reported from almond and peach. The two hazelnut LOX genes showed a similar intron/exon organisation even though, they differ for the size of the first intron which was hypothesized to be involved in the regulation of tissue specific expression. 3’ RACE was carried out in order to characterise the 3’ UTR regions of the two LOX genes. Expression analysis, by Real Time PCR, is in progress in order to compare the expression profiles of the two genes in different hazelnut tissues and in seed samples collected at various developmental stages.

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Cloning and Characterization of Genes Involved in the Production of Secondary Metabolites in *Olea europaea* L.


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Flavonoids and large part of isoprenoids are ubiquitous plants secondary products that have diverse functions in plant physiology and ecology. Among their roles in adaptive responses to environmental challenges, they have also been reported to play an important role in plant-pathogen interaction.

Squalene Synthase -SS-, Farnesyl PyroPhosphate Synthase -FPPS- (isoprenoids pathway) and Chalcone Isomerase -CHI- (flavonoids pathway) genes have been isolated and characterized in olive cultivars autochthonous of Molise. Gene isolation has been carried out through PCR reactions on olive cDNA with degenerated oligonucleotides designed on homologous sequences from other plant species. Olive cDNA was obtained from mRNA extracted from leaves and roots tissues. Full length cDNA sequences were obtained with 3’ and 5’ RACE. Genomic sequences were then amplified and cloned. Since we found evidence of the presence of more than one copy of the CHI and FPPS genes in the olive genome, Southern blotting experiments are in progress to determine the copies number.

The three analyzed genes, showed conserved introns-exons structure when compared with orthologs of *Nicotiana*, *Petunia* and *Arabidopsis*. Re-sequencing of these genes has been performed to develop new SNPs (Single Nucleotide Polymorphisms) to be used for association analysis, cultivar characterization and phylogeny studies. Since we found evidence of the presence of more than one copy of the CHI and FPPS genes in the olive genome, Southern blotting experiments are in progress to determine the copies number.

Furthermore, in an attempt to verify if the studied genes are activated by plant pathogens such as *Verticillium dahliae*, gene expression is being monitored in olive roots after the inoculation with diverse pathogen concentrations.
In situ analysis of specific floral genes expression during in vivo and in vitro reproductive processes in oil palm

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oil palm, somaclonal variation, mantling, floral gene expression

In vitro culture of oil palm (Elaeis guineensis J., Dura x Pisifera) is commonly applied to propagate the most productive F1 elite plants via somatic embryogenesis and to set up new commercially important cultivated area in many Asiatic sub-tropical country.

But is a matter of fact that, particularly in oil palm, in vitro regeneration processes cause large somaclonal variation on the regenerants and may affect vegetative parts of the plant, floral organs and fruits (mantled phenotype) resulting in great crop losses.

In this study we analysed by means of in situ hybridization technique, the pattern of expression of four specific floral genes both in male and female inflorescences of normal regenerated plants and abnormal mantled plants. Furthermore a partial floral-like differentiation in vitro has been observed during the establishment of new oil palm embryogenic culture obtained starting from excised zygotic embryos and extensively studied by means of a cyto-histological approach and in situ investigation.

The floral specific probes, isolated and provided by MPOB (Malaysia Palm Oil Board), were: #327 homologous to the Arabidopsis genes RLK5 (receptor–like protein kinase 5, Walker, 1993), pOP-SFB107 related to Festuca pratensis Xet1 protein (xyloglucan-endotransglycosylase, Vissenberg et al., 2005), pOP-SFB6 and pOP-SFB97 connected to the family of Rho-related proteins (Christensen et al., 2003).

In situ hybridization analysis demonstrated that the probes were all expressed both in male and female inflorescences sharing evidences that there was a stage-specific expression during the in vivo floral development and that there is a constant trend showing always a lot more enhanced pattern of expression in the inflorescences coming from abnormal plants.

Moreover, the clear hybridization signals, for all the probes so far tested, present in the floral-like structures in culture, indicated a deregulated gene expression along the in vitro differentiation processes included somatic embryogenesis.

The overall suggestion coming from the in situ analysis point to the possibility that some of these sequences could eventually be used as predictive markers of abnormality.

STUDIES OF MEIOTIC PROCESS IN OIL PALM (*ELAEIS GUINEENSIS, JACQ.*): A FISH APPROACH

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oil palm, somaclonal variation, meiosis, FISH analyses

*Elaeis guineensis* is a major crop species producing high quality oil used in many foods. The common oil palm is a major crop species producing high quality oil used in many foods. The commercial palms are F$_1$ hybrids between selections with large thick-shelled kernels (*dura*) and small shell-less kernels (*pisifera*). In this monocotyledonous species with a single apical meristem, vegetative propagation (e.g. by cuttings) of elite adult palms has proved impossible, so clonal propagation through tissue culture has been explored for propagation. Extensive research from the1980s has been successful in setting up and optimizing large-scale tissue culture propagation, and plants may be routinely regenerated from cultures initiated from juvenile leaves of adult *tenera* palms, the ortets. However, a proportion of the regenerants, approximately 5–10% of all clonal palms regenerated by *in vitro* micropropagation, shows a flowering abnormality (mantling) that leads to fruit abortion and no yield. The mantled phenotype involves a feminization of both male and female flowers that in oil palm are produced alternatively on the same plants. In abnormal male flowers, stamens develop as carpelloid structures, whilst in abnormal female flowers, the staminodes (vestigial stamens) develop as pseudocarpel structures. In both cases, the petals appear to develop as sepaloid structures; thus, there is a clear similarity with the type B floral mutants identified in *Arabidopsis thaliana* and *Antirrhinum majus* (Coen and Meyerowitz, 1991). In severe cases the flowers are sterile, although lesser affected female flowers may be fertilized to give mantled fruit. These abnormalities are examples of somaclonal variation, that is a multi-cause phenomenon which represent as different pattern of cellular activity can leads to similar phenotypic effects. The mantled character is epigenetic in nature as demonstrated firstly, by the fact that reversion to a normal floral phenotype may occur in the field, secondly, in that weak non-Mendelian transmission of the abnormality occurs via seeds compared with strong transmission through tissue culture (Rival *et al.*, 1997, 1998; Matthes *et al.*, 2001). Recently, Giorgetti et al 2007 have showed that in oil palm chromatin bodies are extruded at meioses from the early meiotic prophase stage nuclei. This phenomenon could represent the release of a surplus of somatic DNA copies to assure single copy DNA pairing. The same of lost DNA sequences being recovered along the seed germination stage. One of the best approaches to study meiosis is FISH analyses that allow the characterization of the time and identity of lost, and recovered, DNA sequences during the meioses. FISH analyses of normal plant in comparison with the mantled phenotype was performed. For FISH analyses we have considered Repetitive sequences as rDNA, Retrotransposable elements both Ty1 or *copia* - and Ty3 or *gypsy*-like. FISH analyses gave evidences that the sequences...
considered showed their localization both on the meiotic prophase chromosomes and on extruded chromatin bodies. More sequences to be probed could shed a better light on the nature of extruded sequences and the postulated genomic surveillance system that is particularly active and important at meiosis, since plants lack a specific germ line. Somatic meiosis, observed in vitro cultures, following the same pattern as seen by the whole DNA decrease in regenerated plants, could represent one of the source of somaclonal variation, particularly if the process of loss and recovery of specific DNA sequences is not perfectly regulated.

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