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Oral Communication Abstract – 2A.01

DEVELOPMENT OF A NEW WHEAT MICROARRAY FROM A TOTIPOTENT CDNA LIBRARY AND IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES FOR POWDERY MILDEW RESISTANCE

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5-azaCytidine, totipotent cDNA, powdery mildew, microarray, durum wheat

In the present work we reported an innovative method for gene discovery based on the use of suitable quantities of 5-azaCytidine in the germination phase of plants to induce the de-methylation of the total DNA. This let to the obtainment of seedlings potentially expressing whole the genes present in the target genome in a early plant stage avoiding plant regulation systems. On this bases a new wheat microarray consisting of 4925 expressed sequence tags, was developed from a totipotent cDNA library and used for the screening of two near isogenic lines, cv. Latino of durum wheat and the line 5BIL-42 susceptible and resistant respectively, to powdery mildew. In order to identify genes involved in resistance responders, the two isogenic lines were infected and grown in greenhouse under controlled condition. Three replicas for each treatment has been carried out. The seedling were grown with a temperature of 22°C under continues light. A single isolate (O2) of powdery mildew was used for inoculation seedlings. Leaves samples were collected after 24, 48 and 72 hours from incubation at 22°C under continues light in the two isogenic lines and immediately frozen at –80°C for RNA extraction.

The RNA samples were extracted and reverse transcribed into cDNA, labelled using two different fluorophores (Cy3, Cy5) and then hybridised simultaneously to each glass slide. Six genes were found differently expressed in two isogenic lines. Search similarity of these sequence in public databases indicated for four sequence a putative function while two were unknown. Physical mapping of the six genes located the accession AJ611689 on chromosome 5BL in the centromeric bin where the resistant *Pm36* gene was previously located, indicating this as the putative resistance gene.

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Oral Communication Abstract – 2A.02

THE X-CHROMOSOME EVOLUTION IN DOMESTIC BOVIDS AS REVEALED BY COMPARATIVE FISH-MAPPING OF CATTLE, SHEEP, RIVER BUFFALO AND HUMAN X-CHROMOSOMES

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X-chromosome, evolution, FISH, gene, cytogenetic map

X-chromosomes of cattle, river buffalo and sheep summarize the three main types of X-chromosomes present in bovids (123 species). Indeed, this chromosome is submetacentric in cattle, acrocentric in river buffalo and acrocentric with distinct p-arms in sheep. C-banding technique revealed that while cattle and sheep X-chromosomes are C-band negative, the river buffalo X-chromosome shows a prominent C-band at the centromere and a proximal additional C-band. Detailed cytogenetic maps have revealed that complex chromosome rearrangements differentiated the evolution of this chromosome in bovids.

In this study 15 animals from cattle (Agerolese breed), river buffalo (Italian Mediterranean breed) and sheep (Laticauda breed) were used to perform blood cell cultures to obtain R-banding chromosome preparations to be used for FISH-mapping applications. BAC-clones from both ovine and bovine libraries were used for the FISH. At least 30 metaphases for animal and probe were studied under a fluorescence microscope connected with a digital camera. Twenty-one new loci were assigned to the X-chromosome of these bovid species, noticeably extending the cytogenetic map in this chromosome. This allowed us to show the most advanced and detailed physical maps today available in this chromosome species. A direct comparison among X-chromosome of cattle (BTA-X), river buffalo (BBU-X), sheep (OAR-X) and humans (HSA-X) was performed revealing that: (a) BTA-X and BBU-X have the same gene order and differentiated by a centromere transposition or centromere repositioning with loss of constitutive heterochromatin (from buffalo to cattle); (b) OAR-X differentiated from BTA-X/BBU-X by at least four transpositions including a centromere transposition or repositioning; (c) a large and distal chromosome segment of BTA-Xq has been conserved entirely during the evolution not only in bovids but also in humans, excluding a centromere repositioning present in both sheep and human Xs.

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Oral Communication Abstract – 2A.03

GENOMIC COMPARISON OF THE SEQUENCES CONTRIBUTING TO THE 3D STRUCTURE OF THE IgH 3' REGULATORY REGION (»30 kb) IN VERTEBRATES: CONSERVATION OF A LARGE PALINDROMIC STRUCTURE WITH A POLYMORPHISM OF THE INTERNAL ENHANCER

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Regulatory Region, Genomic interspecies survey, 6 Kb palindromic structure, Enhancer Tetraplex 3D conformation

Background: Three-dimensional (3D) conformation and relative arrangement of chromosomes in the nucleus has a major role in controlling gene-expression programs. Regulatory Regions as LCR perform their activity through different mechanisms by means of secondary structures and epigenetic changes. The mechanisms involved for these 3D remodeling are mediated by consensus sequences of DNA and formation of complexes DNA-protein able to localize specific genomic regions inside nuclear factories (Eskiw et al., *C S H Symp Quant Biol* 2011, volume 75: 501-506). The Immunoglobulin heavy chain (IgH) 3' Regulatory Region (3'RR), plays a crucial role in immunoglobulin production and B cell maturation. In humans, there are 2 copies of the 3'RR. Rodents have only one copy of 3'RR with an extra enhancer probably acquired by a rodent–lineage specific duplication event (D'Addabbo et al. BMC Evol Biol. 2011 Mar 15;11:71-83).

Results: To determine the evolutionary conservation and transformation of the main structures of IgH 3'RR we compared the genomic organization in vertebrates. We found that in the 8 species in which the whole genomic region was included in a fully assembled contig (mouse, rat, dog, rabbit, panda, orangutan, chimpanzee, and human), the main elements showed synteny and a highly conserved sequence. The wide 3'RR (»30 Kb in human) bears in all species a large palindromic sequence, consisting in two ~3 Kb complementary branches spaced by a ~3 Kb sequence always including the HS1.2 enhancer. The maintenance of the palindrome was despite an inter-specific divergence at sequence level. Another relevant result concerns human polymorphism of the HS1.2 enhancer, associated to immune diseases in our species. We detected a similar polymorphism in all the studied Catarrhini (a primate parvorder). The polymorphism consists of multiple copies of a 40 bp element, separated by stretches of Cytosine. The number of duplicates is up to 12 in chimpanzees, 8 in baboons, 6 in macaque, 5 in gibbons, and 4 in humans and orangutan. We confirmed specific binding of these elements to nuclear factors. The in *silico* prediction of "tetraplex" structures in 3'RR enhancers suggested to study in vitro these DNA sequences for the formation of 3D tetraplex. Our analysis includes UV spectroscopy, CD and NMR on HS1.2 polymorphic "Tetraplex" predicted region.

Conclusions: We want to remark that the palindrome is retained in evolutionary distant species. It suggests pressures for the maintenance of two self-matching regions driving a hairpin structure. The conservation of the palindromic structure and the primates polymorphic feature of HS1.2 show the relevance of these structures in directing and modulating the Ig production through the formation of three-dimensional organization. The hypothetical “tetraplex” formation and its variability for different alleles of the HS1.2 enhancer is another possible feature confirming the regulatory activity mediated by secondary structures of DNA.

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Oral Communication Abstract – 2A.04

KNOX LOST THE MEINOX: ALTERNATIVE SPLICING OF THE ARABIDOPSIS *KNAT1/BP* GENE PRODUCES AN ISOFORM THAT LACKS THE PROTEIN-PROTEIN INTERACTION DOMAIN

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Transcription factors, Arabidopsis thaliana, plant architecture, functional genomics, alternative splicing

Knotted1-like homeobox (KNOX1) transcription factors belong to the three amino acid loop extension (TALE) superclass of homeodomain proteins in higher plants and are essential for proper formation and maintenance of the shoot apical meristem (SAM). Within the TALE, KNOX proteins are closely related to myeloid ecotropic viral integration site (MEIS) proteins in humans, owing to a conserved N-terminus region. This domain, called MEINOX after KNOX and MEIS, defines this subclass of the TALE family. Protein-protein interactions with a second group of TALE proteins, the BEL-like homeodomain (BLH) family, modulates KNOX1 nuclear localization and target selection. These interactions depend on the KNOX MEINOX domain, and each homeodomain of the two proteins binds to target DNA as a protein heterodimer. Such regulatory interactions are reminiscent of those between different TALE proteins in animals, indicating that they might have an ancient origin.

KNOX1 transcription factors exert their role in maintaining indeterminate cell fate through the modification of multiple hormonal pathways to integrate developmental signals at the SAM. *KNOX1* transcription factors are also expressed in the stem where they regulate plant architecture and lignin deposition. Mutations in *KNAT1/BREVIPEDICELLUS* (*KNAT1/BP*), one of the four Arabidopsis *KNOX1* genes, result in plants with shorter internodes, downward pointing siliques, altered intra-bundle cell identity and altered lignin deposition. Recently, *KNATM*, a novel Arabidopsis *KNOX1* gene that encodes a MEINOX domain but lacks the homeodomain was identified and shown to regulate KNAT1/BP.

In this study, we identify and characterize a novel isoform of the KNAT1/BP transcription factor that arises from an event of alternative splicing and produces a novel protein (BPhox) that encodes a homeodomain but lacks the MEINOX domain. Expression patterns, intracellular localization, protein-protein interactions of this novel isoform have been characterized. Overexpression of the two isoforms under the control of constitutive/inducible promoters and complementation analyses were carried out to unravel the function of BPhox in plant development.

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Oral Communication Abstract – 2A.05

TRANSCRIPTOME CHARACTERIZATION OF PHENOTYPIC PLASTICITY IN *VITIS VINIFERA*

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Vitis vinifera, transcriptome, phenotypic plasticity, differential gene expression

Phenotypic plasticity, the ability of an individual to change its phenotype in response to changes in the environment, is common among plants owing to their sessile lifestyle. It has been deeply studied in plants, both for its agronomical significance as well as its ecological and evolutionary implications. However, mechanisms determining plastic changes are still mostly unclear especially for plants cultivated in open fields, where the simultaneous challenge of different environmental signals leads to complex responses in terms of gene expression, metabolic rearrangements and epigenetic mechanisms.

Vitis vinifera spp is one of the most plastic plants known, a single genotype being able to produce berries with different quality, thus different wine qualities, depending on the micro-environment where it is cultivated. Moreover, plastic responses in grapevine are one of the causes of excellent-to-poor wine vintages.

In this study, plastic rearrangements of the transcriptome of a single clone of *Vitis vinifera* cv Corvina (48) were analyzed. Berries of different developmental stages were harvested from 11 vineyards during 3 consecutive years. A total of 171 hybridization were performed using a NimbleGen microarray system designed on the new V1 grapevine genome annotation.

More than 5% of the whole Corvina transcriptome was estimated to be used for plastic reorganization of the gene expression during berry development. Plastic dynamics were also assessed in light of seasonal changes, highlighting transcripts expressed during standard meteorological trends and those expressed under unfavorable weather conditions. Differential gene expression was further correlated with metabolic and enological data as well as central micro-environmental and agricultural features revealing how human-imposed conditions rather than natural environment might trigger plastic reactions in ripe berry transcriptome.

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Oral Communication Abstract – 2A.06

INTRIGUING ISSUES FROM A HIGLY DUPLICATED GENOME: AN EXAMPLE FROM TRANSCRIPTION FACTOR GENE PARALOGS

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Transcription factors, whole genome duplication (WGD), paralogs

Gene duplication followed by functional diversification of the duplicated genes (paralogs) is a major driver of evolution. There are evolutionary scenarios where paralogs are significantly over-retained following whole genome duplication events (WGDs) but at the same time they may exhibit lower retention rates after smaller scale duplications.

The model diploid plant *Arabidopsis thaliana* underwent several rounds of WGDs, followed by reduction and reshuffling of the gene content. Therefore, an all-against-all protein sequence similarity search allowed the identification of all the possible pair-wise similarities between genes, classifying structurally related ones into networks of paralogs. The data have been organized in a user-friendly web accessible database.

To further exploit these data, we focused on transcription factor genes. In fact, since the presence of widespread intra-genome duplications, together with the loss of gene copies, the interpretation and the study of the evolution of transcription factor gene families is very complicated and this threats the role of this genome as a reference in plant comparative genomics. Moreover, due to their key roles in gene regulation, transcription factor are among the best examples of dosage-sensitive genes.

Our effort required to overcome one of the major limitation to studying TFs in *A. thaliana*, i.e. the lack of a reliable and unique annotation, a challenge that is compounded by the presence of many dedicated databases and several methods for the identification of genes encoding DNA-binding domains. In a first step, we focused on well-known TFs collections to validate and integrate their data. Then, we performed a deep investigation on transcription factors organization within the *A. thaliana* genome, via the analysis of paralogs.

Our approach provides support to the classification of TFs in *A. thaliana* and represents a step forward to understand TF family organization and evolution.

The analysis here presented confirms the usefulness in exploiting the collection of network of paralog genes in *A. thaliana*, since it permits an appropriate investigation of gene families and reveals interesting issues concerning this reference plant genome.

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Oral Communication Abstract – 2A.07

GENOME SEQUENCE AND ANALYSIS OF THE TUBER CROP POTATO

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Solanum tuberosum, genome sequencing, genome annotation, haplotype diversity, tuber biology

Potato (*Solanum tuberosum L.*) is the world's most important non-grain food crop and is central to global food security. It is clonally propagated, highly heterozygous, autotetraploid, and suffers acute inbreeding depression. Here we use a homozygous doubled-monoploid potato clone to sequence and assemble 86% of the 844-megabase genome. We predict 39,031 protein-coding genes and present evidence for at least two genome duplication events indicative of a paleopolyploid origin. As the first genome sequence of an asterid, the potato genome reveals 2,642 genes specific to this large angiosperm clade. We also sequenced a heterozygous diploid clone and show that gene presence/absence variants and other potentially deleterious mutations occur frequently and are a likely cause of inbreeding depression.

Gene family expansion, tissue-specific expression and recruitment of genes to new pathways contributed to the evolution of tuber development. The potato genome sequence provides a platform for genetic improvement of this vital crop.

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Oral Communication Abstract – 2A.08

ΔNP63α AND YB1: A FUNCTIONAL INTERACTION CONTROLLING CELL PROLIFERATION AND MOTILITY

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P63, cold shock domain protein, PI3K signaling, cell proliferation, cell migration

TP63, a member of the p53 gene family, can be expressed as six different protein isoforms. ΔNp63α is the predominantly expressed p63 isoform in squamous epithelia and is restricted to cells with proliferative potential while it is downregulated in cells undergoing terminal differentiation. ΔNp63α plays a critical role in the morphogenesis of organs/tissues developing by epithelial-mesenchimal interactions such as the epidermis, teeth, hair and glands. Herein, we present evidences that the Y box-binding protein-1 (YB-1), a member of the cold shock domain superfamily of proteins, interacts with ΔNp63α but not with the smaller ΔNp63γ isoform. YB-1 is a transcriptional/translational factor involved in cell proliferation, migration and transformation but the molecular mechanisms governing the involvement of YB-1 in these processes are still unclear.YB-1 shuttles between the nucleus and cytoplasm playing different and even conflicting functions according to its sub-cellular localization. We present data showing that enforced expression of ΔNp63α induces YB-1 nuclear localization and accumulation. In the nuclear compartment ΔNp63α and YB-1 activates the expression of the catalytic subunit of the PI3K kinase (PI3KCA) thereby inducing Akt1 phosphorylation and activation of a pro-survival pathway. Furthermore, our data suggest a role for ΔNp63α in the control of cell motility and adhesion through interaction with YB-1. We also present data showing that ΔNp63α and YB1 colocalize in proliferative cells of epithelia. Our data implicate the existence of a ΔNp63α-dependent mechanism governing cell proliferation and migration through the physical association between ΔNp63α and YB-1, a newly isolated p63 molecular partner.

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Oral Communication Abstract – 2A.09

THE ROLE OF ta-siRNAs IN MAIZE PLANT DEVELOPMENT

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Ta-siRNAs, plant development, Dicer-like 4, maize

Plants produce a variety of small RNAs (sRNAs), including microRNAs (miRNAs), small interfering RNAs (siRNAs) and *trans*-acting siRNAs (ta-siRNAs). Members of these classes act as endogenous regulators of gene activity and target the expression of different genes**.** Ta-siRNAs arise from specific *TAS* loci and in *Arabidopsis* different TAS gene families have been characterized, among them the *TAS3* family, whose products target the *AUXIN RESPONSE FACTORs* (*ARFs*) genes.

The ta-siRNAs biosynthesis starts with the transcription of the *TAS* genes, whose transcripts are processed by miRNA. The cleavage products are bound by a SUPPRESSOR OF GENE SILENCING (SGS3), converted into dsRNA by an RNA-DEPENDENT RNA POLYMERASE (RDR6) and subsequently processed by the DICER-LIKE4 (DCL4) into the 21 base-pair siRNAs. The 21 base-pair siRNAs guide the cleavage of target transcripts. For the *TAS3* ta-siRNAs, the miRNA-directed cleavage of the target transcripts is mediated by a specialized ARGONAUTE, AGO7.

ta-siRNAs belonging to the TAS3 family play a crucial role in plant development. In *Arabidopsis* they are involved in the juvenile-to-adult vegetative phase change, in the development of lateral roots and in the establishment of the leaf polarity.

In maize the *leafbladeless1* and *raggedseedling2* genes have been identified as the orthologues of *SGS3* and *AGO7* respectively. In this species, as well as in rice, this pathway is also involved in the meristem maintenance and formation. For a deeper comprehension of the role exerted by ta-siRNAs in the maize plant development we are performing a functional study of the *shootmeristemless1* (*sml1*) gene, whose product corresponds to the *Arabidopsis* DCL4. Depending on the genetic background, mutations in the *sml1* gene cause the complete deletion of the apical portion of the shoot and of the shoot meristem or lead to the formation of plants with several developmental abnormalities.

Our analysis is focused at different processes, such as the formation of seminal and lateral roots, the establishment of leaf polarity, the transition from juvenile to adult leaf and the organization of leaf epidermis. Data will be presented on the comparison between wild-type and mutant tissues at different developmental stages.

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Oral Communication Abstract – 2A.10

CANDIDATE-GENE RESEARCH TO UNDERSTANDING THE ROLE OF GENETIC DIVERSITY IN THE ADAPTIVE RESPONSE OF MEDITERRANEAN PINES

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Adaptive evolution, neutrality tests, environmental associations, candidate genes, Mediterranean pines

Combining coalescence modelling, neutrality tests and environmental correlations, demographic history and non-neutral patterns of evolution in candidate genes related to drought stress and secondary compounds were investigated in two closely-related Mediterranean conifers, *Pinus pinaster* Ait. and *Pinus halepensis* Mill. Amplicons covering candidate genes were sequenced in a sample from the full range of these two species. Higher levels of nucleotide diversity in candidate genes for drought response were present in *P. pinaster* than in *P. halepensis*, despite its narrower range in the Mediterranean. Differences across species were also reflected in the haplotype distribution for each tree species, with *P. pinaster* showing many different haplotypes at similar frequencies and *P. halepensis* showing fewer haplotypes with only one that is common or even fixed. The low levels of nucleotide diversity in Aleppo pine are more noticeable in its western distribution where most genes were fixed or almost fixed for particular haplotypes, a probable consequence of long-range colonization of the Western Mediterranean from ancient Aleppo pine populations in the easternmost edge of its current distribution and a more acute impact of the Ice Ages in this range of the species. Molecular analyses also revealed intense and relatively recent bottlenecks in Aleppo pine as well as a time of split between North-African and Iberian populations of the species well predating the Last Glacial Maximum albeit not as old as the one estimated for maritime pine. In contrast, maritime pine seems to harbor large amounts of diversity for these genes due to a more stable demography; in addition, because of its more mesic distribution, higher environmental heterogeneity would have resulted in contrasted selective pressures that may have increased general levels of diversity at candidate genes. Using a wide range of neutrality tests, we found some of the candidate genes studied to evolve in non-neutral patterns. Interestingly, two of them showed statistical correlation with temperature variables, in particular with extremely high or low temperatures, and may constitute valuable tools for monitoring adaptive genetic diversity in these two Mediterranean pines. Our study shows that the use of complementary approaches can help capturing different aspects of the evolutionary processes that govern molecular variation at both intra- and inter-specific levels.

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Oral Communication Abstract – 2A.11

AKTIP, A NEW LAMIN INTERACTING PROTEIN IS INVOLVED IN TELOMERE METABOLISM AND DNA REPLICATION

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Telomeres, replication, lamins

Proper telomere maintenance is a crucial process needed to protect the genome against instability and telomere dysfunction that has been linked to tumorigenesis and premature aging. Driven by results assigning a telomeric role to its *Drosophila* homologue *peo*, we have collected a set of data on a new human gene that can be linked to telomere metabolism, AKTIP. AKTIP down regulation triggers proliferation impairment, premature senescence and DNA damage response activation. AKTIP KD causes telomere dysfunction, as indicated by the presence of DNA damage foci at telomeres (TIFs) and by that of aberrant telomeres in AKTIP KD $p53^{-/-}$MEFs, including multiple telomeric signals at the ends of chromosomes, also known as fragile telomeres, indicative of telomere replication impairment. The mechanistic role of AKTIP appears, indeed, to be linked to replication: AKTIP can interact with DNA and with crucial components of the replisome (RPA and PCNA), furthermore, AKTIP KD cells display an intra-S block. A particularly seducing aspect of AKTIP comes from its localization, characterized by a typical punctate signal at the nuclear rim. This pattern is consistent with the interaction of AKTIP with nuclear lamins, which we have assessed by GST-pull down and mass spectrometry, and also with that with components of the replication forks (e.g. PCNA), which typically situate at the periphery of the nucleus in the final part of S-phase.

Taken together, our data suggest that AKTIP could become a new important player of the mechanistic scenarios of different human diseases linked to “telomeraging” including cancer and laminopathies.

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Oral Communication Abstract – 2A.12

LONG RANGE EFFECTS OF SELECTION FOR BERRY COLOR ON GENETIC DIVERSITY ARE DETECTED IN GRAPE CHROMOSOME 2

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Next generation sequencing, haplotype, grape, SNPs, berry color

Anthocyanin pigmentation in berry skin is a major determinant of grape berry color. Recent investigations provided evidence that a gene cluster of MybA transcription factors, located on the distal part of chromosome 2 at 14.2 Mbp, is responsible for most of the variation in the expression of UFGT, an enzyme critical to anthocyanin biosynthesis, and that color phenotype is due to the combined additive effects of MybA alleles. White berry is a derived trait from the ancestral colored berry state. Despite its importance, no study has yet investigated the long range effects of selection for berry color in chromosome 2.To this end, we compared the genetic structure of a large region of chromosome 2 around the berry color locus in 25 white-skinned and 34 red-skinned grape varieties, arbitrarily sampled to include much of the genetic diversity in the most widely cultivated varieties.

We sequenced 44 amplicons, spaced approximately every 100 kbp from position 14 Mbp to 18.8 Mbp of chromosome 2, identifying 576 single nucleotide polymorphisms (SNPs) and reconstructing the haplotypic structure of this portion of chromosome 2. One haplotype was shared by all white-skinned varieties analysed, and it was also present in the PN40024 line used for whole-genome sequencing. Fourteen white-skinned varieties (56%), were homozygous for this haplotype, while no red variety was homozygous for the derived white haplotype. Nevertheless, the majority of the most cultivated red varieties shared large portions of the distal end of chromosome 2 with white varieties. Specifically, two red-skinned varieties (Tempranillo and Carignan) share most of the amplicons (>80%) with white varieties in both homologous chromosomes, with the exception of the region surrounding the MybA gene cluster. Statistical tests for departure from neutrality detected selective pressure in white varieties only, and showed that strong signature of selection was extending for more than 2 Mbp from 14Mbp to 16Mbp, and some evidence was still present at 18Mbp.

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Poster Communication Abstract – 2A.13

A COMPARATIVE STUDY OF SKELETAL MUSCLE TRANSCRIPTIONAL PROFILES IN TWO CATTLE BREEDS

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Bovine, RNA-seq, skeletal muscle

It is well-esablished that different cattle breeds differ in muscle characteristics due to differences in their physiology. Friesan and Chianina are two cattle breeds selected in the past century for milk end meat traits, respectively. This selection may have influenced muscle features and, consequently, muscle quality. Muscle biological traits are controlled by gene expression and gaining insights with respect to differential expression profiles would provide valuable information to better understand the difference in meat quality. To evaluate whether divergent genetic selection was associated with modified patterns of gene expression in longissimus muscle, transcriptional profiles have been analyzed in LD muscle of both breeds using an RNA-seq approach. RNA-seq is a methodology which allow a quantitative measurement of gene expression through massively parallel RNA sequencing. This methodology allow a comprehensive transcriptome analysis, an accurate measure of individual gene expression as well as detection of rare gene transcripts. The comparison of muscle transcriptional profile revealed significant differences (as astimated DESeq/edgeR packages) in expression of about 800 genes between the two breeds. Further undergoing approaches include resolution of differential espression at transcript isoform level (Cufflinks package). The results reported here will be important to help to identify the genes underlying beef quality traits.

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Poster Communication Abstract – 2A.14

DIFFERENT APPROACHES TO DEVELOP NOVEL MOLECULAR MARKERS FOR SEX DETERMINATION IN *ASPARAGUS OFFICINALIS*

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Molecular markers, sequencing, sex chromosome, A. officinalis

Cultivated asparagus (*Asparagus officinalis* L.) is a dioecious species (2n=2x=20) with a haploid genome size of 1,323 Mb. With respect to sex expression female, male, supermales, andromonoecious and hermaphrodite plants are known. As male plant is agronomically superior respect to female one, a breeding goal is all-male hybrids constitution. In the present study, novel molecular markers have been developed with different approaches, in order to isolate candidate genes associated with sex expression.

In asparagus up to date genetic maps were originally based on isozymes, RFLP, AFLP and RAPD. Using next Generation Sequencing technologies as GSFLX 454 pyrosequencing, thousands of codominant markers (SNPs and SSRs) have been isolated from male and female cDNA libraries. Thus, codominant molecular markers are used to map a large BC_1 population for sex traits. Linkage analysis using MAPMAKER with a LOD score of 3.0 and max distance of 5.0cM provided a map with coverage for 19 linkage groups consisting of 73 markers, starting from a first panel of 144 SNPs. Sex-linked SNPs were identified in linkage group 12 (LG-12). Further experiments are underway to place additional EST-SNP and EST-SSR markers isolated from a BAC library.

The large number of independent shifts between dioecy and hermaphroditism at different times since the origin of flowering plants, makes angiosperms the group of plant useful for testing a general model explaining the evolution of dioecy. In this frame, asparagus is considered a model for studying the evolution of sex chromosomes. Starting from flanking region sequences of the M-locus (the region controlling sexual dimorphism) obtained from three BAC clones (2 from males and 1 from female), three different regions around this site have been identified: one recombinant region between male and female, one non recombinant region and then a male specific region. These sequences were BLASTed against a large asparagus database and novel sex-specific markers (from a region linked 0.25cM to M-locus) have been developed to investigate genes and regulatory networks involved in the origin and early diversification of flowering plants.

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Poster Communication Abstract – 2A.15

MITOCHONDRIAL GENOME OF *ASPLENIUM NIDUS* REVEALS FEATURES HIGHLY SIMILAR TO THOSE OF SEED PLANT mtDNAs

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Plant Mitochondria, Monilophytes, *Asplenium nidus*

Mitochondrial genomes of land plants have been fully sequenced and characterized in several species belonging to the Briophytes (*Marchantia polymorpha* and *Physcomitrella patens*) and Spermatophytes (*Arabidopsis thaliana*, *Beta vulgaris*, *Oryza sativa*, *Brassica napus*, *Zea mays*, *Nicotiana tabacum* and *Triticum aestivum*).

To gain more knowledge on the mitochondrial biogenesis of Monilophytes, we chose plants of a filicale family, the fern *Asplenium nidus*, available at the Botanical Garden of the University of Bari.

The comparison of organization, structure and expression between Spermatophyte mitochondrial genomes reveals several homogeneous features which can be summarized as follows:

i) the presence of repeated sequences,
ii) a heterogeneous structure,
iii) the presence of DNA segments of extra mitochondrial origin (mainly chloroplastic) carrying in some cases active genes (usually for tRNAs),
iv) the editing of transcription products of structural genes,
v) an incomplete set of tRNA genes.

On the contrary not all of these features can be considered peculiar properties of Briophyte mitochondrial genomes, in particular: RNA editing, active in some species but not in all, absence of homologous recombination events; absence of incorporation of foreign genetic information; absence of chloroplast DNA insertions.

Using two differents approach - RAPD PCR and an unusual PCR procedure, we were able to obtain several sequences of the *A. nidus* mitochondrial genome.

The main results obtained from our investigation are the following:

i) the detection of DNA segments of chloroplast origin (*trnA, rps11, rpoB, psbA, rrn16S*)
ii) the identification of complete genes for proteins, rRNA and tRNAs (*nad4L, nad9, atp9, coxIII,rrn26, rrn 5S, trnN, trnR, trnK, , trnP, trnW*);
iii) a couple of inverted repeats although of small size.
iv) Higher transcription editing level respect Spermatophytes

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Poster Communication Abstract – 2A.16

MOLECULAR DISCRIMINATION OF THE GENUS *MENTHA* BY SEQUENCING AND RFLP ANALYSIS OF THE 5S rRNA NON-TRANSCRIBED SPACER (NTS) REGION

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Mentha species and hybrids, 5s rRDNA NTS, PCR-RFLP, molecular fingerprinting

The genus Mentha is of particular economic importance. The development of new methods for the characterization of Mentha species and hybrids is crucial for their unequivocal identification. By amplification of NTS (Not-Transcribed-Spacer) of the 5S-rRNA gene we characterized the major sexual Mentha species and many interspecific hybrids and found a high specific and interspecific variability. Cloning and sequencing of all amplified NTS fragments allowed evaluating similarities and calculate cluster analysis, which confirmed the taxonomic relationship between species and hybrids. In silico and experimental analyses identified specific restriction sites on the amplified 5S-NTS regions facilitating the rapid and unambiguous discrimination of all the different species and hybrids. A specific restriction marker allowed the general characterization of the genus Mentha.

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Poster Communication Abstract – 2A.17

QTLs FOR RESISTANCE TO THE LEAF RUST *PUCCINIA BRACHYPODII* IN THE MODEL PLANT *BRACHYPODIUM DISTACHYON*

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Brachypodium distachyon[0], linkage map, leaf rust, quantitative resistance, QTL

The wild grass *Brachypodium distachyon* (Brachypodium) was already proven as a useful model plant species for temperate cereals, but its potential to study the interactions with economically relevant pathogens remains underexploited. Leaf rust is one of the major fungal diseases affecting temperate cereals, and recently the interaction between Brachypodium and the leaf rust *Puccinia brachypodii* was proposed as a model plant pathosystem. The objective of our study was to identify genomic regions associated with quantitative resistance to leaf rust in Brachypodium. We selected two inbred lines Bd3-1 and Bd1-1 with quantitative differences in their level of susceptibility to *P. brachypodii*, that were crossed to develop an F2 population. A set of 110 F_2 plants were evaluated for their reaction to a virulent isolate of *P. brachypodii* at both seedling and advanced growth stages. To validate the results obtained on the F2 population, resistance levels were quantified in F2-derived F3 families in two independent experiments. Disease evaluations showed continuous, quantitative and transgressive segregation for leaf rust resistance. We applied the AFLP-based technology in Brachypodium, and provide a novel Bachypodium linkage map anchored to its genome sequence. The map, consisting of 203 loci and spanning 811.8 Kosambi cM, included AFLP, SSR, and SNP markers.

Three QTLs of leaf rust resistance were mapped on chromosomes 2, 3 and 4, repeatedly identified across experiments. This study is, to our knowledge, the first quantitative analysis of any trait in Brachypodium, in which we demonstrate that resistance to the *Puccinia brachypodii* isolate is governed by a few major loci with relatively large effects. The results obtained, coupled with the wide range of genomic resources available for Brachypodium, opens new avenues for exploring grass-rust interactions in this model grass.

Co-localization on physical/genetic maps of 10 candidate genes with the QTLs of resistance to leaf rust disease is being done. At the same time, the sequences of the CGs are being characterized in the two parents for further validation and selection. The research is coupled with the study of the organization of barley genes orthologous to the Brachypodium CGs in syntenic genomic regions. The two steps are a starting point towards QTL cloning, to understand mechanisms of quantitative resistance to leaf rust in the model grass, and to export this knowledge to the Triticeae species.

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Poster Communication Abstract – 2A.18

GENETIC DIVERSITY IN *ARTEMISIA PETROSA* SSP. ERIANTHA

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Molecular markers, AFLP, in vitro propagation

Artemisia petrosa ssp. eriantha, a Central Apennines' sub-endemic species, is an aromatic plant used for preparation of herbal medicines, with anti-spasmodic and anti-inflammatory properties, and for the production of a traditional liqueur (genepì). It differs in morphologic characters (length of basal leaves, ear density, length of glandular trichomes) from *A. petrosa* (Baumg.) Jan. ssp. eupetrosa Giac. & Pignatti, which typically grows in the Alps, Central Pyrenees, Carpathians and Balkans, but is absent in the Central Apennines. *A. petrosa* ssp. eriantha has been reported in different locations around the National Park of Gran Sasso and Monti della Laga, and also in the Majella, Sibillini mountains and Maritime Alps.

In the present study AFLP molecular markers were used to assess the genetic diversity in natural populations of *A. petrosa*; gathered on Gran Sasso and Majella mountains. Genomic DNA polymorphisms were studied using six primer combinations. The amplification products were evaluated by capillary electrophoresis and visualized by GeneMapper software 4.0. The data obtained, in the matrix form 1-0 (presence/absence), were examined by statistical analysis.

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Poster Communication Abstract – 2A.19

PHEXPA1 CONTROLS CELL SIZE AND THE TIMING OF AXILLARY MERISTEM DEVELOPMENT IN *PETUNIA HYBRIDA*

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Axillary meristem, cell expansion, cell wall, expansin, Petunia hybrida

Expansins are cell wall proteins required for cell enlargement and wall loosening in many developmental processes. It is thought that the role of expansins may be to dissociate the polysaccharide complex that links microfibrils together, but the mechanism is still poorly defined.

PhEXPA1 is an expansin A gene from *Petunia hybrida*, preferentially expressed in petal limbs where it controls cell expansion and final organ size by preparing the cell wall for the deposition of crystalline cellulose during extension. We analyzed the expression and localization of PhEXPA1, confirming that the protein is localized in the cell wall of expanding tissues. *PhEXPA1* promoter activity was evaluated using a promoter-GUS assay and the protein's subcellular localization was established by expressing a PhEXPA1-GFP fusion protein. To create transgenic *PhEXPA1* overexpressing petunia plants, cauliflower mosaic virus (CaMV) 35S promoter was employed. The constitutive overexpression of *PhEXPA1* in petunia plants significantly affects organ size, and also changes the architecture of the plant by initiating premature axillary meristem outgrowth, indicating that expansin could have a crucial role in plant morphogenesis. Moreover, we evaluated that higher *PhEXPA1* mRNA levels correlated with an increase in expansin activity by extensometer measurements. Fourier transform infrared (FTIR) and chemical analysis were used for the quantitative analysis of cell wall polymers. The alteration of polymer composition in transgenic plants provides a new insight into the role of *PhEXPA1* in cell wall metabolism.

These results support a role for expansins in the determination of organ shape, in lateral branching, and in the variation of cell wall polymer composition, probably reflecting a complex role in cell wall metabolism.

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Poster Communication Abstract – 2A.20

INTERFERENCE OF *LAR* GENES DEPLETES THE SYNTHESIS OF PROANTHOCYANIDINS IN *LOTUS CORNICULATUS* LEAVES

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Proanthocyanidins, RNAi, LAR, Lotus corniculatus

Lotus corniculatus is a useful model system for studying the synthesis of proanthocyanidins (PAs), also known as condensed tannins (Paolocci *et al*., 1999). These flavonoids are relevant to many industrial applications, but they also harbour properties that are of prominent interest to human and animal nutrition and, in general, health. Indeed, PAs help preventing bloating in ruminants when these animals are fed with fresh forage legumes because they increase the rate of rumen bypass proteins, ultimately leading to higher protein assimilation and to better animal performances (Barry and McNabb, 1999)

In a recent study, we cloned the structural genes coding for leucocyanidin reductase (LAR) and for anthocyanidin reductase (ANR). These are the enzymes that lead to the synthesis of catechin and epicatechin, respectively, from the legume species *Lotus corniculatus* (Paolocci *et al*., 2007). Catechins and epicatechins are the building blocks for the synthesis of PA polymers in many crop species. Two *LAR* gene families, *LAR1* and *LAR2*, with an overall nucleotide homology of 60% were recovered. However, *in vitro* evidence showing the possibility of reducing anthocyanidins to catechins has been output only for *LAR1*. Differently from *LAR1*, *LAR2* genes are also very poorly expressed in PA accumulating organs (i.e leaves and stems). To test for the specific relevance of *LAR1* and *LAR2* genes on PA synthesis, *LAR1*- and *LAR2*- based RNAi constructs have been prepared and utilised to transform two genotypes of *L. corniculatus* accumulating different levels of leaf PAs. Preliminary colorimetric and spectrophotometric results show that leaf PA synthesis is severely compromised by both *LAR1* and *LAR2* interfering constructs, regardless of the recipient genotype used. Of note, the down regulation of *LAR1* genes affected also the expression of *LAR2* ones and vice versa, likely because of the partial sequence homology between these gene families. Preliminary qRT-PCR data also suggest that the genes of the flavonoid pathways upstream *LAR1* and *LAR2* are also down-regulated. Together, these data let us to postulate that the down-regulation of the late genes of the PA pathway induces a negative feed-back mechanism on early genes. Detailed metabolic evaluation of all these lines is an ongoing effort in our lab as well as the molecular and metabolic characterization of *Lotus corniculatus* lines expressing *ANR*-based RNAi constructs. This approach should definitely clarify whether a cross-talk between the catechin and epicatechin branches of the PA pathways does exist.

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Poster Communication Abstract – 2A.21

THE TRANSCRIPTION FACTOR *VvMYBPA1* INDUCES A METABOLIC REPROGRAMMING OF FLAVONOIDS IN TOBACCO FLOWERS

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Ectopic expression, LC-ESI-QTOF-MS/MS, Nicotiana tabacum, proanthocyanidin, secondary metabolites

Proanthocyanidins (PAs) are polyphenolic compounds synthesized using anthocyanidins as precursors through one or more enzyme-catalyzed reactions. PAs are secondary metabolites found in a wide range of plants where they play a chief role in protecting them against herbivores, pathogens and abiotis stresses. Thanks to their beneficial properties, both as antioxidants and anticancer agents, there is an increasing interest in promoting the accumulation of these metabolites in food plants. However, PAs are also relevant to ruminant nutrition and animal health because moderate amounts of these flavonoids in the foliage of the forage legumes prevent ruminal bloating, while reducing greenhouse gas emission by livestock and promoting animal tolerance against intestinal parasites. Yet, de novo engineering of PA biosynthesis in forage and food species has proven difficult. Here we test the hypothesis of taking advantage of the anthocyanin pathway to build on PA biosynthesis. To this end, we ectopically expressed in tobacco the gene *MYBPA1,* that in *Vitis vinifera* specifically activates the structural genes of the PAs pathway (Bogs *et al*., 2007). T_0 transgenic *35S:MYBPA1* tobacco lines and their progeny were assayed for flower colour and PAs accumulation. The colour of floral limbs negatively correlated with transgene expression in both primary transformants and their progeny. Spectrophotometric quantification of anthocyanins and PAs as well as qRT-PCR analyses carried out to test the effect of the transgene on endogenous biosynthetic and regulatory genes of flavonoids converge to show that *MYBPA1* prevents anthocyanin formation by channelling all the common precursors toward PA biosynthesis**.**

To glean deeper insight into the metabolic changes triggered by the transgene, we are currently performing LC-ESI-QTOF-MS/MS analyses aiming to specifically compare the profiles of the secondary metabolites in flowers exhibiting different steady state levels of the transgene and, in turn, different levels of anthocyanins and PAs in floral limbs and tubes.

Transformation of *Medicago sativa* is in progress to test the possibility of diverting the flux from the anthocyanidin pathway to PAs also in this species to produce bloat-safe varieties.

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Poster Communication Abstract – 2A.22

MINING MICROSATELLITES IN THE *TUBER MELANOSPORUM* GENOME FOR POPULATION GENETIC ANALYSES

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Tuber, truffles, SSR, genetic diversity, population genetics

The level of genetic diversity and genetic structure in *T. melanosporum,* the most appreciated black truffle worldwide, has been debated for many years. Bertault *et al.* (1998) claimed that *T. melanosporum,* also known as the Perigord truffle, has a low genetic diversity and its populations lack any genetic structure. As a consequence, the different bouquet exhibited by different truffle populations has been primarily attributed by these authors to ecological rather than genetic determinants. The absence of genetic structure within *T. melanosporum* populations has been however questioned by Murat *et al.* (2004) and, more recently, by Riccioni *et al.* (2008) who identified a significant level of genetic differentiation among populations of this species. However, all these studies relied on a limited set of molecular markers that also showed a low level of polymorphism. Thanks to the availability of the whole genome assembly of *T. melanosporum* (Martin *et al.,* 2010) it is now possible to identify a plethora of markers for population genetic analyses.

To this end, this study aimed at annotating and characterizing SSR (simple sequence repeats) loci in the genome of *T. melanosporum.* The *T. melanosporum* genome is rich in microsatellite loci with 22,425 SSRs. Mono-nucleotide SSRs are the most frequent motifs. SSRs were found in all genomic regions although they are more frequent in non-coding regions. Sixty out of 135 PCR-amplified mono-, di-, tri-, tetra, penta, and hexanucleotides were polymorphic (44%) within black truffle populations and 27 were randomly selected to analyse 139 *T. melanosporum* specimen collected in France, Italy and Spain. The number of alleles per locus varied from 2 to 18 and the expected heterozygosity from 0.124 to 0.815. One hundred and thirty-two different multilocus genotypes out of the 139 *T. melanosporum* isolates were identified and the genotypic diversity was high (0.999).

In conclusion, the SSRs characterized in this study were highly polymorphic and our results showed that *T. melanosporum* is a species with an important genetic diversity, which is in agreement with its recently uncovered heterothallic mating system (Rubini *et al*., 2011).

The availability of highly polymorphic molecular markers coupled to a large-scale truffle collection, representative of the species distributional range, present in our labs will allow us to gain insight into the extent and geographical distribution of genetic variability among black truffle populations. In turn, robust population genetic analyses will allow us to test the hypothesis that not only environmental but also genetic factors play a critical role in shaping the aroma of the black truffle.

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Poster Communication Abstract – 2A.23

IDENTIFICATION AND CHARACTERIZATION OF SEX-RELATED GENES IN *TUBER MELANOSPORUM* GENOME

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Tuber, truffles, mating type, pheromones, signal transduction

The genome of *T. melanosporum,* the symbiotic ascomycete producing the most valuable black truffles, has been recently sequenced providing researchers with an exceptional opportunity to learn more about the biology of this fungus (Martin *et al.*, 2010). The genetic traits controlling the symbiotic process, the metabolic pathways responsible for aroma production as well as mechanisms of mating and fruit body development are fundamental aspects not yet fully disclosed in this fungus. More specifically, understanding the reproductive mode that governs the production of truffles has been a long-standing dilemma for mycologists (Rubini *et al.,* 2007). The availability of *T. melanosporum* genome has however provided us with genetic tools to identify, and study the organization of, the mating type (*MAT*) genes and conclude this fungus is heterothallic, i.e. an obligate outcrossing species (Martin *et al.,* 2010; Rubini *et al.,* 2011a; 2011b).

In heterothallic ascomycetes the *MAT* genes encode transcription factors that regulate expression of genes involved in signaling among compatible sexual partners: the so called pheromone/receptor system. Binding of mating type specific pheromones (α-factor and a-factor) to their specific G protein-coupled receptors (STE2 and STE3) triggers a signal transduction pathway that induces the expression of several genes involved in many processes such as cell fusion, fruit body development and meiosis (Kim *et al.,* 2002).

In this study an inventory of candidate sex-related genes was compiled by performing *in silico* analysis of *T. melanosporum* genome. This analysis revealed that most of the key components of the fungal pheromone-dependent signaling pathways are conserved in *T. melanosporum* including the pheromones and their receptors. Furthermore, several *T. melanosporum* homologs of genes controlling karyogamy, meiosis and fruit body development in other ascomycetes were identified.

T. melanosporum mycelial strains of opposite mating types have been also isolated and *in vitro* dual cultures of mycelia of opposite mating type set to study the nutritional, environmental and genetic components that drive the transition between vegetative and reproductive phase.

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Poster Communication Abstract – 2A.24

CHARACTERIZATION OF *MEDICAGO TRUNCATULA* LHA GENE AS MULTIFUNCTIONAL OXIDASE INVOLVED IN THE BIOSYNTHESIS OF HAEMOLYTIC SAPONIN

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Medicago truncatula, saponin, lacking haemolytic activity (LHA), oleanolic acid

Saponins are a group of glycosidic compounds present in several plant species whose aglycone moieties are formed by triterpenoid or steroidal skeletons. In spite of their importance as antimicrobial compounds and their possible benefits for human health, knowledge of the genetic control of saponin biosynthesis is still lagging behind. Here we report on the identification of a cytochrome P450 gene (LHA) involved in saponin synthesis in *M. truncatula* using a combined approach of genetics and biochemistry. Genetic loss-of-function analysis and complementation proved that LHA is responsible for an early step in the saponin pathway, as the mutants reported were unable to produce haemolytic saponins (*lha*) but only synthetized soyasaponins. In vitro enzymatic activity assay indicates that LHA catalyzes oxidation of β-amyrin and erythrodiol at C-28 position, to yield oleanolic acid. Transcriptome changes in the mutant lacking haemolytic activity showed a modulation in the main steps of triterpenic saponin biosynthetic pathway: squalene cyclization, β-amyrin oxidation and glycosylation.

This report provides a first evidence for a key oxidative step in haemolytic saponin biosynthesis.

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Poster Communication Abstract – 2A.25

ROLE OF A NOVEL *KNOX1* TRANSCRIPTION FACTOR OF *MEDICAGO TRUNCATULA* IN THE DETERMINATION OF LEAF MORPHOLOGY

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Transcription factors, M. truncatula, leaf development, functional genomics, alternative splicing

In higher plants, KNOX1 homeobox transcription factors play a critical role in compound leaf development. The presence of *KNOX1* in developing leaves characterizes compound-leaved versus simple-leaved species, where *KNOX1* genes are expressed only in the shoot apical meristem (SAM) and permanently down-regulated during leaf development. It has been recently shown that modifications of *KNOX1* gene expression, through changes of cis-regulatory properties, represent the main evolutionary step of leaf morphology complexity.

In simple-leaved species, KNOX1 are essential for proper formation and morphogenetic activity of the SAM, whereas in most of compound-leaved species they exert their role in leaf dissection through the maintenance of a transient indeterminacy and morphogenetic activity at the marginal blastozone. In the inverted repeat-lacking clade (IRLC) of legumes, an alternative pathway, involving the Arabidopsis LEAFY (LFY) orthologs - UNIFOLIATA (UNI) of pea and SINGLE LEAFLET1 (SGL1) of *M. truncatula* - was proposed to act in the formation of compound leaves.

In order to explore the role of KNOX1 transcription factors in the development of trifoliate compound leaves in *Medicago truncatula,* a model species for forage legumes, six *MtKNOX*s have been identified and characterized in our lab.

Here we present the molecular and functional characterization of *MtKNOX6*, a homologue of Arabidopsis *SHOOTMERISTEMLESS* (*STM*) that is located in a separate branch of the phylogenetic tree with respect to the strictly STM-like proteins. *MtKNOX6* expresses in developing leaves, in the SAM and during inflorescence development, is regulated through alternative splicing and the translated proteins retain the ability to functionally interact with the BEL protein PNY/BLH9 to enter the nucleus. Transgenic lines that overexpressed *MtKNOX6* in the heterologous system *Arabidopsis thaliana* did not phenocopy Arabidopsis plants that overexpressed *STM*, that suggesting different biochemical and molecular properties of *MtKNOX6* with respect to *STM*. Also, overexpression of either *STM* or *MtKNOX6* in *M. truncatula* did not trigger the same developmental alterations, that confirming the data obtained in Arabidopsis. Two *mtknox6* knock-out lines were isolated in collaboration with The Samuel Roberts Noble Foundation (US) and their characterization is in progress.

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Poster Communication Abstract – 2A.26

IDENTIFICATION OF A *MEDICAGO TRUNCATULA* GLUTATHIONE S-TRANSFERASE GENE ESSENTIAL FOR ANTHOCYANIN ACCUMULATION

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Medicago truncatula, anthocyanin, glutathione S-transferase

Anthocyanins and proanthocyanidins (PAs) are flavonoids that share large part of their biosynthetic pathways. In a screening of a *Tnt1* transposon tagged *Medicago truncatula* population two indipendent mutants lacking anthocyanin pigmentation in the leaves were identified. The observed phenotype co-segregated with an insertion in a glutathione S-transferase (*MtGST*) gene in R1 progenies derived from both mutants. Homozygous mutated plants did not show *MtGST* expression as per real time RT-PCR analysis.

The importance of GSTs for anthocyanins transport has been demonstrated in different plant species where mutations in the encoding genes led to the reduction in anthocyanins accumulation and pigment mislocalisation. Indeed, it has been proposed that GSTs either aid in cellular detoxification by catalysing the conjugation of glutathione to anthocyanins and other flavonoids or act as flavonoid-binding matrix to protect these compounds from oxidation and favour their long-distance transport. Nevertheless, GSTs are one of the molecular actors associated with vacuole sequestration of anthocyanins and PAs. In this regard, spectrophotometric assays highlighted the absence of anthocyanins in the homozygous mutated plants and a reduced accumulation in the heterozygous compared to the wild type. Conversely, no qualitative alteration in proanthocyanidins presence was detected by 0,2% DMACA staining of seeds and glandular trichomes in the mutants.

Using both molecular and computational approaches we show that *MtGST* is the ortholog of *AN9* in *Petunia* and *Bz2* in maize. Further studies are ongoing to estabilish *MtGST* role in anthocyanin transport and sequestration in *Medicago truncatula* as well as to assess its role, if any, in PAs accumulation.

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Poster Communication Abstract – 2A.27

TOMATO PLANTS OVEREXPRESSING CRYPTOCHROME 2 REVEAL MODIFIED EXPRESSION OF CHLOROPLAST GENOME

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Photoreceptors, chloroplast, tiling array, tomato, gene expression

Tomato is a major food commodity especially in the Mediterranean countries. It represents a source of fibres, carbohydrates, proteins, lipids, minerals as well as of several vitamins and anti-oxidants, namely lycopene, that plays an important role in the prevention of some diseases like prostate cancer. The relative concentration of all these substances is finely regulated by biochemical processes that occur during ripening and which are related to genetic and environmental cues, as light and temperature. The ability to perceive and transduce light is important for normal growth and development of many organisms. Higher plants are able to monitor the environmental light conditions by using multiple wavelength-specific photoreceptors including red/far-red absorbing phytochromes and blue/UVA absorbing cryptochromes and phototropins. Light changes activate, by photo-perceptive proteins, a cascade of biochemical and molecular processes which change the physiological state of plants. Some of these changes may regard the chloroplast genome expression in photosynthetically active tissues. The cryptochromes, UV-A/blue light photoreceptors, play an important role in the monitoring, capturing and transmitting the light stimuli. In tomato four cryptochrome genes have been identified and characterized so far: CRY1a, CRY1b, CRY2 and CRY3. CRY2 gene has a central role in tomato plant development. Its over-expression in the transgenic CRY2-OX line, is of high relevance for the overproduction of anthocyanins and chlorophyll in leaves and fruits, suggesting its role in promoting the photosynthetic performance.

In this study we analysed the effects of CRY2 over-expression on chloroplast genome transcription in tomato, by using a genome tiling array. High density arrays containing 90k 35mer oligonucleotide probes were produced using CustomArray technology. The tiling array consisted of 30 nt overlapping probes, covering the entire chloroplast genome. Total chloroplast RNA extracted from wild type and CRY2-OX plants was used to hybridize the microarrays.

This study allowed to identify genes differentially expressed between CRY2-OX and wild type plants, in particular those involved in photosynthetic activity. We also found genes with complex transcriptional architecture, where transcripts corresponding to different parts of the same gene are detectable at different levels. The analysis also allowed to map the positions of 3' and 5' UTRs of coding genes. Potentially microRNAs could be also identified.

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Poster Communication Abstract – 2A.28

A DEEP INSIDE PLANTS GENE STRUCTURE PLASTICITY: PSY GENE FAMILY AS A CASE STUDY

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Carotenoid pathway, Psy gene family, synteny, wheat, intron loss

Carotenoids are isoprenoid pigments essential for photosynthesis and photoprotection in plants. Their biosynthesis begins with the formation of the 40 – carbon backbone, phytoene, a step mediated by phytoene synthase (PSY). This gene is thought to be rate–limiting enzyme of the carotenoids pathway; PSY is nuclear encoded by a small gene family consists of three paralogous genes (PSY1-3) that have been widely characterized in rice, maize and sorghum for which genes structure are quite conserved except for PSY3 in sorghum and *Brachypodium*, both characterized by the loss of one intron and the fusion of two exons. In wheat, for which yellow pigment (YP) content is extremely important regarding the flour colour, only PSY1 had been extensively studied because of its association with reported QTLs for YP and only partial information are available for PSY2. Here, we report the isolation of bread wheat PSY3 from Renan BAC library. The main difficult is due to polyploidy, the presence of duplicated genes and the lack of sequence information for wheat, reason why we used *Brachypodium* as a model genome for the *Triticeae* to develop COS (Conserved Orthologous Set) primers to easily identify BAC clones harbouring the gene of interest. At the same time, new assemblies of sequences from 454 Chinese Spring sequencing project led us to derive PSY3 consensus sequence and develop primer pairs to get the full – length gene sequence from isolated BAC clones. Based on the obtained PSY3 homeologous BAC sequences, we analysed their structure: wheat PSY3s are characterized by the loss of the first two introns due to the presence at the deletion breakpoints of repeated inverted motifs that have generated during DNA replication a loop in the DNA strand giving rise to an event of replication slippage resulting in intron loss. Wheat PSY3s were mapped on the long arm of chromosome group 5, while PSY1s and PSY2s are localized, respectively, on the long arm of chromosome group 7 and on the short arm of group 5. Based on paleogenomic analysis of the PSY gene family in cereals we suggest that they originally derived from an ancient WGD event specific to the Monocots. Since there is not evidence about PSY3 gene expression, we evaluated the expression of homoeologous copies for each PSY genes in several tissues, such as developing grains, leaves and roots. Structural, functional and evolutionary data obtained fror the PSY gene family will be presented and discussed.

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Poster Communication Abstract – 2A.29

ENERGY BIOGENESIS: HOW TO COORDINATE TWO GENOMES

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Mitochondrion, regulation

Mitochondria are considered to be the powerhouse of the cell. In mitochondria the degradation of the carbohydrates is coupled with the synthesis of high-energy molecules such as ATP, which powers up the vast majority of chemical reactions of the cell.

The biogenesis and function of mitochondria is the result of coordination between the nuclear genome, where there is more than 95% of mitochondrial genes, and the mitochondrial genome.

This coordination is particularly rigorous also because the protein complexes both of respiratory chain (OXPHOS) and mitochondrial ribosomes are assembled according to well-established stoichiometric relationships.

In higher eukaryotes has been reported that this type of coordination is achieved mainly at the transcriptional level. Various experimental observations suggest the presence of a fine-tuned communication between mitochondrial and nuclear genomes that results in the interdependent expression of OXPHOS genes and ribosomal genes encoded by mtDNA and nuclear genome.

Having previously identified, by using a series of bioinformatics analysis, a group of five DNA motifs that, for the distribution and frequency in putative regulatory regions of OXPHOS, TCA, and ribosomal genes, could be regarded as excellent regulatory sequences, we decided to validate this analysis by means of the One-Hybrid Assay.

The One-hybrid assay was performed using two different DNA motifs as bait, the Site II motif (ref) and the Ac/tTGT motif. This analysis showed that some proteins are able to interact "in vivo" with those motifs. We have identified a transcription factor belonging of the family AP2/ERF/B3 (AtERF#115) that binds Site II motif. The transcription factor is part of the regulative cascade of ethylene, this finding could help explain the mechanisms by which ethylene is able to influence the respiration (e.g. climacteric).

We have also identified two transcription factors belonging to the family of bZIP (AtbZIP18 and AtbZIP52), when we used the Ac/tTGT motif as a DNA-bait. The transcription factors are involved in the process of growth and development of the plant.

The family of bZIP transcription factors is required in the process of growth and development of the plant and therefore it can be possible that AtbZIP18 and AtbZIP52 may be involved in response to those processes.

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Poster Communication Abstract – 2A.30

FUNCTIONAL CHARACTERISATION OF SHORT VEGETATIVE PHASE BY A CHROMATIN IMMUNO-PRECIPITATION SEQUENCING APPROACH

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SVP, AP1, ChIP-seq, floral transition, floral meristem

In plants organs are formed post-embryonically from populations of undifferentiated cells called meristems. In flowering plants like Arabidopsis thaliana during the vegetative phase the primordia that derive from the shoot apical meristem (SAM) develop into leaves. The change to the subsequent generative phase is called floral transition, which is regulated by multiple flowering pathways that are controlled by environmental and endogenous cues. During the floral transition the SAM is transformed into an inflorescence meristem (IM). The Arabidopsis IM is an indeterminate meristem and develops in a spiral manner multiple determinate floral meristems (FMs) that produce a precise number of floral organs arranged in a whorled pattern.

SVP is a key MADS-box transcription factor for Arabidopsis development since it acts both during vegetative and reproductive phases where it plays different roles probably by interacting with different partners to regulate specific sets of target genes. In fact, whereas SVP functions as a repressor of floral transition during the vegetative phase, it works as floral meristem gene during reproductive phase.

Here we report the identification of genome wide binding sites of SVP using the ChIP-seq technology that consists in ultra-high throughput Solexa (Illumina) sequencing of DNA samples obtained by Chromatin Immune-Precipitation. We studied the binding behavior of SVP during two distinct developmental phases: the vegetative and reproductive phase. Furthermore, by combining the ChIP-seq data with tiling ATH microarray expression analysis and qRT pcr approach, helped us to identify subsets of genes that are directly regulated by SVP during the two distinct phases of development. Finally we compare the genome-wide direct target genes of SVP with that of FLC, a closely related transcription factor that also represses the transition to flowering and with that of AP1, a MADS-box factor which is, together with SVP, a key player during floral meristem development. We detect clear similarities and important differences in the direct target repertoires that are also tissue specific. This analysis allowed us to identify new pathways that are regulated by SVP in vegetative and reproductive tissues and to investigate the dynamics of genome wide interactions of a transcription factor during different phases of development.

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Poster Communication Abstract – 2A.31

DE NOVO SEQUENCING AND TRANSCRIPTOME ANALYSIS OF *KALANCHOE* TO IDENTIFY PUTATIVE GENES INVOLVED IN EPIPHILLY

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Blast2GO, K. x houghtonii, pyrosequencing, epiphilly

In nature, asexual reproduction plays an important adaptive role.. The process is widespread to several families (e.g. *Crassulaceae, Graminaceae, Liliaceae*). Among Crassulaceae, vegetative vivipary (epiphilly) has been reported on leaves, stems and flower stalks of many Kalanchoe species. In *K. xhoughtonii*, a tretraploid interspecific hybrid, viviparous plantlets are formed on leaf margin notches in response to a long day photoperiod and their appearance follow a basipetal fashion.

Recently, next generation sequencing (NGS) technologies provide new options to understand complex phenomena in non-model species developing affordable tools for functional genomics.

With the purpose of identify and characterize genes putatively involved in vegetative vivipary and in leaf morphogenesis, *de novo* sequencing and analysis of *K. xhoughtonii* trascriptome were performed by 454 pyrosequencing technology.

Total RNA was isolated from apical meristems (comprehensive of leaf primordia) and from leaf at diverse stages of development (0,5 , 1 and 3 cm), using RNeasy Plant Mini kit (QIAGEN) to prepare two full length cDNA libraries (labelled M_ and S_Library), respectively. Library normalization and pyrosequencing with the GS FLX 454 Titanium system was performed by Eurofins MWG-Operon (Ebersberg, Germany).

454 reads from the two libraries were independently assembled using MIRA Assembler. The 578.856 and 593.130 HQ reads obtained with two half-plate run from the M_library and S_Library were assembled in 120.650 and 138.816 unigenes (contigs plus singletons), respectively. The unigene sequences were annotated by BLASTx versus non-redundant protein database (nr) with Blast2GO bioinformatics tool (http//:www.blast2go.org). In both libraries, the species that provided most of the top BLAST hits was *Vitis Vinifera* (about 25.600 and 23.061 genes, respectively).

The ORFPredictor tool (http://proteomics.ysu.edu/tools/OrfPredictor.html) : computed that about 99,5% contigs in both libraries correspond to protein-coding genes.

The Gene Ontology annotation and "Augment Annotation by ANNEX" function, to refine annotations, was done using Blast2GO. 54,08% and 57,6% contigs were annotated in the S_ of, and the M_library, respectively. The GO terms obtained by the annotation procedure were mapped to a plant specific GO-Slim to simplify and highlight differentially expressed GO classes. KEGG orthologs and biological pathways were assigned to unigenes.

Overall, differences were displayed among GO classes between the two libraries. The GO terms analisys was resulted 836 and 854 genes in the transcription factor activity (level 3 of molecular function) of M and S_Library, respectively. In particular putative genes and gene family involved in vivipary and in leaf morphogenesis were identified.

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Poster Communication Abstract – 2A.32

THE SPINACH *LHCB1* MULTIGENE FAMILY. WHY MULTIPLE GENES FOR ISOFUNCTIONAL PROTEINS?

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Light harvesting protein, plant adaptation, Spinacia oleracea, multigene family, genome walking

Conversion of light energy in higher plants is carried out by two electrically connected photosystem units, PSI and PSII, containing pigment-protein complexes that act as antennae to harvest solar light energy. The light harvesting complex II (LHCII), associated with PSII, contains three highly homologous chlorophyll-*a**b*-binding proteins (Lhcb1, Lhcb2 and Lhcb3), which can be assembled in both homotrimers and heterotrimers. Generally Lhcb1 and Lhcb2 are encoded by multigene families, whose members are variable in number from plant to plant, and whose different function is yet to be clarified.

We have identified in spinach leaves the full-length cDNAs corresponding to three isoforms of Lhcb1 polypeptides, whose isoforms appear to be differentially expressed in response to long-term white light exposure (1). The three Lhcb1 mature polypeptides are highly homologous to each other, sharing very high identities (97-98%). Only the Lhcb1.1 polypeptide shows an amino acid substitution having a clear functional meaning. It corresponds to a Thr3>Ser substitution which was found to affect phosphorylation level of Lhcb1 polypeptides, a crucial step in the State Transition process (2).

In order to sequence the regulatory regions of the spinach *Lhcb1* genes, we have developed a suitable Genome Walking method which allows the contemporary analysis of members of multigene families (3, 4). By this approach we identified two additional *Lhcb1* genes, not previously hypothesised on the basis of cDNA and proteomic analysis. Gel shift assays of identified regulatory motifs showed the different activity that distinct regulatory motifs may have in the expression of members of the gene family (5). These results, together with the phylogenetic analysis of the *Lhcb1* families, sustain the hypothesis of a role of members of the multigene families and their regulatory regions in the adaptive response of plants to different light conditions.

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Poster Communication Abstract – 2A.33

DEVELOPMENT OF A *VITIS VINIFERA* miRNA MICROARRAY PLATFORM

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Plant miRNAs, microarrays, Vitis vinifera

MicroRNAs (miRNAs) are small non-coding RNAs that play key roles in various biological processes. To gain insight into miRNA function, it is fundamental to obtain a high-resolution profile of their spatiotemporal distribution.

In order to characterize miRNA expression patterns in several tissues and developmental stages in *Vitis vinifera* cv. Corvina we have designed and validated a *Vitis vinifera* miRNA microarray based on the Agilent technology and established a complete protocol from smallRNA extraction to data analysis.

The design consists of 480 probes corresponding to mature miRNAs and miRNA star coming from public databases and/or in-house produced deep-sequencing data. Each probe has been replicated 10 times and for each probe a double mismatch control was included to assess hybridization specificity.

A good reproducibility of data has been obtained between technical and biological replicates. Data obtained with Agilent platform has been also compared with Combimatrix microarray platform. This tool will allow to perform a comprehensive miRNA expression atlas that will constitute a fundamental basis for define miRNAs function in grape.

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Poster Communication Abstract – 2A.34

PERFORMANCE ASSESSMENT OF DIFFERENT MICROARRAY GRAPE DESIGNS

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Vitis vinifera, ROC, sensitivity ,specificity, significantly differentially expressed genes

The recent development of transcriptomic approaches based on Next Generation Sequencing is gaining popularity as they provide a genome-wide, precise, quantitative measure of gene expression. However microarray still are a valuable tool for differential gene expression studies as they are cheap, easy to manage and to analyze. In this work we have assessed the performances of two microarray platforms (Combimatrix and Nimblegen) and two strategies of probe design (single or multiple probes per transcript) in gene expression analysis across two *Vitis vinifera* berry developmental stages. The same samples were analyzed by RNASeq analysis using an Illumina GAIIx sequencer. In order to assess sensitivity and specificity of the four microarray designs in detecting significantly differentially expressed genes, assuming RNASeq data as reference, Receive Operating Characteristic (ROC) analysis has been performed. Such analysis showed that, disregarding the microarray platform used, array designs with multiple probes per transcripts allow to detect an higher number of differentially expressed genes and exhibit a better agreement with RNASeq data. Moreover the analysis performed showed that sensitivity of the four microarray designs in regard to RNASeq data augment with the increase of expression levels detected by the Next Generation Sequencing (NGS) approach. This data suggest that most of the differences in detecting differential expression between the two approaches is due to the lower sensitivity of microarray for low expressed genes. In order to validate the results and explain discrepancies among the approaches, a set of genes whose expression is in agreement or disagreement among microarray and RNASeq, has been tested by qPCR analysis.

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Poster Communication Abstract – 2A.35

A MULTIDISCIPLINARY APPROACH TO IDENTIFY THE GENETIC DETERMINANTS OF FLAVONOID CONTENT IN GRAPES

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Vitis vinifera, flavonoids, biochemical and transcriptional analysis, QTLs analysis

Anthocyanins and flavonols are among the most abundant flavonoids in grapes and they are synthesized starting from the aminoacid phenylalanine via the well characterized flavonoid biosynthetic pathway. They are important for the quality of grapes and wines since they contribute to their colour, taste and health properties. Although the genetic and biochemical bases of the biosynthesis of these compounds and to lesser extent of its regulation have been recently characterized also in grapevine, the molecular reasons of the varietal variability in terms of quality and quantity are still unclear.

To shed light on this issue we have characterized a mapping population derived from the cross Syrah x Pinot Noir by integrating metabolic, genetic and transcriptional sources of information.

Berries of 170 F1 individuals of the cross were harvested at technological maturity in three different seasons and analyzed for anthocyanins and flavonols content. QTL analysis for these traits identified several QTLs on the available dense genetic map of the cross with different effects and stability among seasons.

The analysis of flavonols allowed also the selection of two groups of individuals having either very low or very high content of these compounds in the berry skin. Gene expression profiling of these two groups by means of microarrays, identified a large set of transcripts differentially modulated between the groups.

Co-localization of some of these transcripts with the QTL regions highlighted in the QTL analysis is helping in the selection of the best candidate genes for further characterization.

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Poster Communication Abstract – 2A.36

DE NOVO RECONSTRUCTION OF THE *VITIS VINIFERA* CV. *CORVINA* TRANSCRIPTOME

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De novo assembly, Vitis vinifera cv. Corvina, transcriptome, next-generation sequencing, gene expression profiling

The widespread adoption of NGS technologies has created in recent years an unparalleled inflow of sequence data, which can be leveraged to obtain clear information on the genetics and transcriptome of various organisms. In agronomics, these new methods can be used to gather information on cultivars of sequenced organisms. In particular we were interested in identifying putative specific genes represented in *Vitis vinifera* cv Corvina and not in the sequence grape clone (Pinot Noir 40024). These genes could be of interest in order to investigate the molecular bases of specific quality characteristics. To this end, we have pooled RNA of *Vitis vinifera* cv. Corvina samples taken from 45 different tissues and/or development stages, and sequenced it with an Illumina HiSeq platform. We obtained more than 100 million reads, which were filtered and assembled with the Velvet/Oases software suite. To facilitate the assembly, we provided the software with the alignment of the reads against the *Vitis vinifera* 12X genome (performed with the TopHat software). The reconstructed transcripts were compared to known genes already annotated and ESTs, to identify potential genes specific of this cultivar. Moreover, in order to estimate the expression levels of these genes, we performed another sequencing experiment encompassing five developmental fruit stages, with three biological replicate each. Using Bowtie and Cufflinks we calculated gene expression levels and identified putative novel genes which are differentially modulated during berry development and withering.

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Poster Communication Abstract – 2A.37

EXPLORING THE MYB-bHLH-WD REGULATORY COMPLEX OF THE FLAVONOID PATHWAY IN *VITIS VINIFERA*: THE bHLH SIDE

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Grape, flavonoids, bHLH, transcription factor, Petunia

The regulation of the flavonoid pathway has been extensively studied in many plant species. It occurs mostly via the control of the coordinate expression of structural biosynthetic genes by a complex of R2R3 MYB transcription factors, MYC-like basic Helix-Loop-Helix (bHLH), and WD40 proteins. In *Vitis vinifera,* a small set of MYB transcription factors have been characterized and their ability to regulate the synthesis of different flavonoid end-products like anthocyanins or proanthocyanidins have been shown to be strictly dependent on a bHLH partner. Recently, the partial characterization of two grape bHLH factors (VvMYC1 and VvMYCA1), putatively involved in flavonoid regulation in berry has been described.

By exploring the last released grape genome prediction we found another gene that we named VvJAF13, coding for a bHLH protein with high similarity to known bHLH factors regulating flavonoid synthesis in other species. Molecular analysis of VvJAF13 revealed that it displays all the basic features for participating to the MYB-bHLH-WD regulatory complex and to be a candidate regulator of the flavonoid pathway. *VvJAF13* has been isolated from *Vitis vinifera* cv Corvina berry cDNA and over-expressed in the *Petunia hybrida an1* mutant, defective in a bHLH factor needed for anthocyanin production in petals. Moreover, grape *VvMYC1* has been also isolated and over-expressed in the same Petunia mutant line. Phenotypic and molecular analysis of transgenic plants showed that only *VvMYC1* was able to complement the *an1* mutation by restoring the wild type flower pigmentation, while *VvJAF13* was not. Interestingly, however, the plants over-expressing *VvJAF13* lost also their residual petal pigmentation and displayed completely white flowers. On the basis of these results the possible role of *VvMYC1* and *VvJAF13* in the regulation of grape flavonoid biosynthesis is inferred.

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Poster Communication Abstract – 2A.38

TRANSPOSITION MEDIATED DECONSTRUCTION OF A (PALEO)POLYPLOID GENOME

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Genome evolution, paleopolyploidy, resistance genes, grapevine, Vitis vinifera

Plants have followed a reticulate type of evolution and taxa have frequently merged via allopolyploidization. The polyploid structure of sequenced genomes has been frequently proposed, but the chromosomes belonging to putative component genomes are difficult to identify. The grapevine chromosomes are, evolutionary, stable structures, existing in triplicate copies.

We focused our investigation on the grapevine *Nucleotide Binding Site* (*NBS*) gene family in order to better understand mechanisms underlying the evolution of the grapevine genome.

We show that, in grapevine, i) helitrons have significantly contributed to *NBS* genes transposition; ii) *NBS* gene cluster similarity indicates the existence of two groups of chromosomes that may have evolved independently.

Time estimation indicates that grapevine component genomes may have fused around 60 mya, having had at least 40-60 mya to evolve independently. Chromosome triplets consist of two Va and one Vc chromosomes, as expected from tetraploid and diploid conditions of two component genomes. The hexaploid state could be derived from allopolyploidy, or the Va and Vc component genomes were separated in the same nucleus before fusion, as known for Rosaceae species. Chromosome number variation in the Vitaceae and related families, and the gap between the time of eudicot radiation and the age of Vitaceae fossils, are accommodated in the frame of our hypothesis.

In conclusion, two groups of grapevine chromosomes seem to have evolved independently, opening the possibility that a tetraploid and a diploid genomes merged to generate the extant hexaploidy state of grapevine.

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Poster Communication Abstract – 2A.39

CHARACTERIZATION AND STUDY OF *VITIS VINIFERA* L. SIRTUIN GENES

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Sirtuin, NAD+-dependent deacetylases, Vitis vinifera, SRT1, SRT2

The sirtuin/Sir2 (Silent information regulator 2) family of NAD+-dependent deacetylases and mono-ADP-ribosyltransferases plays an important role in several cellular processes including gene silencing, cell cycle regulation and life span extension in yeast and animals. Despite the recent explosion in the number of reports on sirtuins in fungi and animals, just few works dealing with plant sirtuins can be retrieved from literature and few is known about their function in plants. Compared to other eukaryotes, plants have relatively fewer *SIR2* related genes and just two putative SIR2 family proteins, SRT1 a protein SIRT6/SIRT7-like and SRT2 a protein SIRT4-like, can usually be found in plant genomes. Recently, two putative sirtuin genes were identified also in the grapevine annotated genome. Starting from the predicted coding sequences present in the database, we have been able to obtain two truly expressed coding sequences from the start to the stop codon for both sirtuin genes that were named *VvSRT1* and *VvSRT2*. The search for the expressed coding sequences was performed by comparing the predicted sequences with the recently available grape RNAseq database with the aim to develop the primers to be used in reverse trascriptase PCR reactions to amplify the genes of interest. Finally, in order to better understand the physiological role of both sirtuins, we investigated the expression of these genes in young leaves, mature leaves, and berries sampled at different growing stages. In leaves, usually it has been observed that *VvSRT1* is less expresses than *VvSRT2*, moreover in young leaves *VvSRT2* showed the higher expression during setting while in mature leaves during the flowering time. No particular variation have been observed concerning *VvSRT1*. In berries the two genes showed more similar expression level, and they showed the highest expression during the flowering time. Finally, the expression of *VvSRT2* in berries is smaller than in leaves.

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Poster Communication Abstract – 2A.40

ROLE OF VvMYB14, A NOVEL R2R3 MYB FACTOR, IN THE WOUND RESPONSE AND IN THE REGULATION OF STILBENE BIOSYNTHESIS IN GRAPEVINE

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Stilbene synthases, R2R3 MYB, downy mildew, abiotic stresses

Stilbene synthases (STSs) are a class of enzymes belonging to the general CHS type III polyketide synthase family involved in the last step of the biosynthesis of stilbenes. These enzymes, and their main products resveratrol or pynosylvin are detectable in only a limited number of unrelated plant species, including grape, and accumulate in response to biotic and abiotic stresses. Despite numerous studies that have been performed on the accumulation, metabolism and biological properties of resveratrol, little is known about the transcriptional regulation of this pathway. As reported in a previous contribution, based on a whole transcriptome sequencing by mean of next generation sequencies thechnology (NGS), we identified a candidate R2R3-MYB transcription factor (TF) which shows an expression pattern similar to that observed for STSs and which could be involved in the regulation of stilbene biosynthesis in grape. This R2R3 MYB factor was designated as *VvMYB14*, based on homology with the *AtMYB14* R2R3 MYB factor. Analysis of *VvMYB14* expression in grape leaf discs treated with biotic (downy mildew infection) and abiotic stresses (wounding and UV-C exposure) known to be involved in the transcriptional activation of *STS* genes, showed a close correlation between the pattern and timing of expression of selected *STS* genes and TF. Using a Dual Luciferase Reporter Assay System in transiently transformed grapevine cells, this TF demonstrated to increase stilbene synthase promoter activity.

Here we present the next steps in the characterization of *VvMYB14*. We focused our attention on the wound response extending the expression analysis also to the other R2R3 MYB TFs known to be involved in the regulation of the flavonoid synthesis. Furthermore we report the first results confirming *in planta* the role of *VvMYB14* as a *VvSTS* trans-activator. We developed a transgenic grapevine hairy root system for testing the effect of both silencing and over-expression of *VvMYB14* on the response of *VvSTS* expression. Preliminary results indicate that roots in which *VvMYB14* has been silenced, show significantly reduced levels of *VvSTS* transcription following the application of the wound stress. Further experiments are now underway to clarify the role of VvMYB14 in the regulation of both the stilbene synthase pathway and genes belonging to the general phenylpropanoid pathway in grapevine.

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Poster Communication Abstract – 2A.41

IN THE *turf* MUTANT OF SUNFLOWER, A TRANSPOSON INSERTION IN A *CYCLOIDEA* GENE (*HaCYC2c*) CHANGES THE FLORAL SYMMETRY AND FERTILITY OF RAY FLOWERS

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Floral symmetry, cycloidea-like genes, Helianthus annuus, transposon, reproductive organs

Floral symmetry appears to be controlled by the same fundamental set of related genes in many distantly related taxa, suggesting a common genetic basis for the trait (Jabbour et al., 2009). *CYCLOIDEA* (CYC)-like genes have been reported to be involved in flower symmetry regulation in various plant species. *CYC* of *Antirrhinum* was the first gene isolated, and thereafter the most extensively studied (Jabbour et al., 2009). It belongs to the plant-specific gene family encoding TCP transcription factors, which share a conserved basic helix–loop–helix TCP domain (Martin-Trillo and Cubas, 2010). Recent studies investigating the role of *CYC* homologs in Brassicaceae, Leguminoseae and Asteraceae flower development support independent recruitment of *CYC* in establishing bilateral symmetry within core eudicots (Preston and Hileman, 2009). The inflorescence of sunflower (*Helianthus annuus* L.) is heterogamous with zygomorphic ray flowers located in the outermost whorl of the head and actinomorphic disk flowers arrayed in arcs radiating from the center of the head. The ray flowers are sterile; disk flowers are hermaphrodite, carrying both male and female organs. The *tubular ray flower* (*turf*) mutant is characterized by a change from a zygomorphic corolla to a nearly actinomorphic tubular-like corolla of ray flowers that also achieves the ability to differentiate fertile stamens and ovules (Berti et al., 2005). The recessive *turf* mutation is found to be unstable, spontaneously reverting to a wild-type or nearly wild-type phenotype (Fambrini et al., 2007). Here, we demonstrate that the mutant phenotype is due to a transposon insertion in the class II *TCP* gene *CYCLOIDEA/TEOSINTE BRANCHED1 (CYC/TB1)*, *HaCYC2c* (Chapman et al., 2008). In heterozygous progenies, the mutant phenotype co-segregate with the transposon insertion. Perfect transposon excision, generates reversion of the mutant to wild type phenotype. By contrast, imperfect transposon excision can generate stable mutant types. It is likely that in ray flower, the *HaCYC2c* gene carries a dual role controlling the corolla symmetry and repressing genes involved in stamen and carpel development. New mutant phenotypes were also detected within the heterozygous reverted progenies, and a gene tagging approach could be employed to isolate the affected genes.

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Poster Communication Abstract – 2A.42

MOLECULAR CHARACTERIZATION OF THE WIDER EUROPEAN *CYNARA* COLLECTION BY MEANS OF MARKERS

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AFLP, ISSR, Simple Sequence Repeats, germplasm

The wider *Cynara* germplasm collection has been build up from former germplasm collections present in Italy, France, and Spain, in the frame of the 'CYNARES' project, sponsored by the AGRI GEN RES Community Programme (European Commission, Directorate-General for Agriculture and Rural Development, under Council Regulation (EC) No 870/2004). The full global artichoke collection include germplasm from all the four artichoke typologies (Violet de Provence, Catanese, Spinoso Sardo, Romanesco), for a total of 151 accessions. A core collection was analyzed for morphological and biochemical traits, while the whole collection was characterized at the molecular level to assess the diversity present, to measure the genetic distance among accessions and to cluster them. In the present paper are reported the results of molecular analyses. SSR, ISSR and AFLP markers were used, for a total of 2403 loci on 543 genotypes. Genomic DNA was extracted from plant leaves and shared among partners; each partner analysed the whole collection using different markers. The total data were used to detect several genetic information such as: Nei's genetic distance, level of polymorphism, observed and expected heterozygosity, presence of private alleles, polymorphism information content (PIC) of each marker. The different marker typologies were also analysed separately. Finally, accessions were clustered and discriminant analysis was undertaken to detect the correct assignment of each genotype with respect to country of origin and artichoke typologies. The majority of genetic diversity was within accession. The Nei's genetic distance is able to cluster together all the accessions with cardoon germplasm; but on the other hand, are located far apart in the dendrogramm accessions called with the same names and obtained by different Institutions; this highlight the not proper Cultivars definition, over years of cultivation in various locations.

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Poster Communication Abstract – 2A.43

INSIGHT INTO THE ORGANIZATION OF THE *CYNARA CARDUNCULUS* GENOME

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Artichoke, Cynara cardunculus, NGS sequencing, genome organization, ripetitive sequences

Deep sequencing offers new possibilities to improve knowledge on genome composition producing a huge amount of sequence information in a short time. We used Illumina platform to explore artichoke genome with the aim of characterize in particular its repetitive component, highlighting remarkable features and generating information for further research applications.

Globe artichoke (*Cynara cardunculus* var. *scolymus*) is an important Mediterranean crop largely cultivated in the area from where it originated.

In this study we provide a first report on the organization of the artichoke genome using Illumina technology, which allowed to obtain a 2.3 x coverage of the variety Brindisino genome. Reads were assembled into contigs and characterized. A short insert plasmid library was constructed from the same genotype, and produced about 1700 clones sequenced using Sanger technology, for a total length of 2.74 Mbp. Moreover, twelve BAC clones belonging to a random sheared library were also sequenced and annotated.

All these sequences were BlastN and BlastX searched against the non redundant nucleotide and protein GenBank databases and against transposable elements databases. Pairwise BlastN comparison of each sequence was performed against all sequences to determine the genome repetitiveness. *Copia*, *Gypsy* and LINE were the most abundant retrotransposable elements; CACTA repetitive elements were also numerous in the artichoke genome. However, a large fraction of the repetitive component seems to be unknown.

A comparison with the repetitive fraction of the genome from other *C. cardunculus* taxa (i.e. wild and cultivated cardoon) was performed.

Results regarding repetitive component of artichoke genome generates important insight in its local organization and will serve in both theoretical and applied research, e.g. to improve strategies for genetic and physical mapping and for the development of molecular markers. Moreover, it represents a prerequisite for the annotation steps in sequencing projects.

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Poster Communication Abstract – 2A.44

TOWARDS THE CONSTRUCTION OF OLEAREP: A DATABASE OF REPEATED SEQUENCES OF *OLEA EUROPAEA* L.

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Repeated sequences, retrotransposons, genome sequencing, next generation sequencing

Improved knowledge of genome composition, especially of its repetitive component, generates information that is of importance in both theoretical and applied research, such as for improving strategies for genetic and physical mapping of genomes and for the discovery and development of molecular markers. Moreover, knowledge of genome composition is a prerequisite for the annotation steps in sequencing projects.

Despite the importance of olive as a crop, studies on structural genomics of *Olea europaea* are rare. In particular, the repetitive component of olive genome has been studied mostly at cytogenetic level, evidencing the occurrence of tandem repeats in centromeres and telomeres. Concerning transposable elements, only a few sequences have been isolated and characterized unitil now.

With the aim of producing a database of repeated sequences to be used for annotation in sequencing projects, we have sequenced 1.2x genomic DNA of olive, cv. Leccino, using the Illumina NGS technique.

We have analysed 1.8 Gb (25,000,000 of 75 nt-long Illumina reads) of sequences of genomic DNA, corresponding to 1.2x coverture. These sequences were assembled using mainly the CLC Bio Workbench 5.0 software following different procedures. In a first assembly, using default parameters, we obtained 105,507 contigs (N_{50} = 252). Of these, 3474 contigs were longer than 500 bp and were annotated against NCBI databases and databases of repeated sequences. On the whole, we identified 581 LTR retrotransposon fragments (350 *Copia*-like, 231 *Gypsy*-like), 28 non-LTR retrotransposon fragments, 14 fragments of unidentified retrotransposons, 76 DNA transposons fragments, 22 putative helitron fragments. 1312 contigs did not show any similarity to known sequences.

In other experiments, the pool of Illumina reads was splitted into 8 or 16 portions covering 0.15x or 0.075x each and assembled separately; the resulting contigs were assembled at their turn using CAP3 assembler obtaining 3254 and 3114 supercontigs. All supercontigs and contigs were masked against the previously obtained *Olea* database. This allowed to identify 306 supercontigs, specific to these assembly procedures and representing mostly retrotransposons and repeated sequences.

We are now sequencing and annotating a number of BAC clones from a library BAC of *O. europaea* cv. Leccino, to identify complete repeated sequences and implement the database.

This database will first applied to the annotation process of the olive genome, that is being subjected to complete sequencing.

Research work supported by MIPAAF, Project OLEA-Olive Genomics and Breeding.

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Poster Communication Abstract – 2A.45

MOLECULAR ANALYSIS OF GENE EXPRESSION LEVELS IN *POPULUS* SPP. TRANSGENIC LINES

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Transgenic poplars, cry gene, transgene copy number, real time PCR

Insect-tolerant poplars have been obtained using several types of insecticidal genes coding for *Bacillus thuringiensis*-toxins. In transgenic plants, transgene copy number can greatly affect the expression level and genetic stability of the target gene, making estimation of transgene copy numbers an important area of genetically modified plant research.

In this study, *Populus alba* and *P. tremula* x *P. tremuloides* transgenic lines, obtained via *Agrobacterium*-mediated transformation, carrying *cry1Ab* and *nptII* genes in the T-DNA region, were investigated by PCR and Real Time PCR (RT-PCR) analysis to estimate the transgene copy number as well as expression of the inserted gene in transgenic poplar, respectively. The plants were vegetatively propagated in growth chambers over 2 years. Ten individuals from each clone were planted in containers with "forest soil", and grown in a climate chamber.

All lines contained one copy of *cry* gene and two of them showed that the copy number was different for the *cry1Ab* and *nptII* genes, suggesting rearrangements or multiple but incomplete copies of the transferred DNA. The copy number was concordant among the 3 individuals of each lines analysed and with those determined from the same transgenic lines kept in micropropagation for 2 years. The transcript levels from both genes were determined in 3 individuals for each line growing in climatic chambers. High levels of mRNA expression were detected with respect to the stable endogenous *actin* gene for both transgenic lines. Comparing the transcript level of inserted genes among lines, a significant low level of *nptII* gene ($p = 0.005$) in the line carrying 3 copies was observed.

The evaluation of the copy number of the inserted genes has indicated their stability after 2 years of micropropagation. The lower expression level of the *nptII* inserted gene in one line could suggest that factors like position effects or DNA rearrangements lead to differential expression.

The screening of the transcriptomic variations in transgenic plants carrying the *cry* gene and the comparison with position effects or DNA rearrangements is in course. The final aim is to unravel possible pleiotropic transcriptomic effects following *cry* gene expression in *P. alba* and *P. tremula* x *P. tremuloides* transgenic lines.

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Poster Communication Abstract – 2A.46

CENTROMERIC SEQUENCES IN THREE POPLAR SPECIES

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Centromeric sequences, P. trichocarpa, P. deltoides, P. nigra

The evolution of centromere structure in plants is far to be clarified. It is mainly composed of tandem repeated sequences and retrotransposons, mainly of the *Gypsy* superfamily. Usually, centromeric repeats are species specific, hence they can be used also to study evolutionary relationships between species.

Though the genome of *Populus trichocarpa* has been entirely sequenced, the structure of centromeres has received little attention. We searched for putative centromeric satellites in the poplar genome using the TandemRepeat Finder software. We identified two types of putative centromeric repeats. The first type, whose consensus sequence is 107 bp long, should allow the identification of the centromere position in 9 over 19 chromosomes forming the haploid complement. The second, a consensus sequence 142 bp long, should identify the centromere of 6 chromosomes. No putative centromeric repeats were found in the remaining 4 chromosomes, probably because of underrepresentation of repetitive sequences in the currently available poplar genome sequence.

We overlapped a map track of putative centromeric repeats for each chromosome with the distribution of retrotransposons along poplar chromosomes. In all chromosomes in which the centromere position seemed to be identified, there was a significant overlap between the putative centromeric position and the accumulation of full-length *Gypsy* retrotransposons, suggesting the association between centromeric repeats and these retroelements. It is however to be recalled that the definition of the centromere position requires biochemical and cytological validation, for example by BAC in situ hybridization.

The presence of two different centromeric repeats in two groups of chromosomes should be related to an ancient interspecific hybridization occurred during *P. trichocarpa* evolution.

We also performed slot blot experiments in other two poplar species, *P. deltoides* and *P. nigra*, using the two *P. trichocarpa* putative centromeric repeats as probes. Such repeats occur also in these two species. Moreover the 142 bp long sequence shows high similarity to a 145 bp tandem repeat sequence isolated by Rajagopal et al. (1999) in another poplar species, *P. ciliata*, that was described as putatively centromeric.

On the whole, the results indicate that the two putative centromeric sequences can be very useful for studying the evolution of the poplar genus.

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Poster Communication Abstract – 2A.47

ANALYSIS OF LTR RETROTRANSPOSONS IN THE GENUS *POPULUS*

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Repeated sequences, retrotransposons, poplar, next generation sequencing

Retrotransposons are a ubiquitous component of plant genomes. They are especially abundant in species with large genomes. *Populus* species have relatively small genomes for which large genomic resources are available, however studies focused on poplar retrotransposons dynamics are rare. With the aim to study the retrotransposon component of the poplar genome, we have scanned the complete *P. trichocarpa* genome sequence in search of full-length LTR retrotransposons, i.e., retrotransposons characterised by two long terminal repeats at the 5' and 3' ends.

A computational approach based on detection of conserved structural features, on building multiple alignments, and on similarity searches allowed to obtain a database of 1,479 putative full-length LTR-retrotransposons. Ty1-*copia* elements were more numerous than Ty3-*gypsy*. However, many LTR-retroelements were not assigned to any superfamily because lacking of diagnostic features and non-autonomous. LTR-retrotransposon remnants were by far more numerous than full-length elements, indicating that during the evolution of poplar, large amplification of these elements was followed by DNA loss. Within superfamilies, Ty3-*gypsy* families are made of more members than Ty1-*copia* ones. Retrotransposition occurred with increasing frequency following the separation of *Populus* sections, with different waves of retrotransposition activity between Ty3-*gypsy* and Ty1-*copia* elements. Recently inserted elements appear more frequently expressed than older ones. Finally, different levels of activity of retrotransposons were observed according to their position and their density in the linkage groups.

To analyse the occurrence of diversity in the retrotransposon pool of different poplar species, a Illumina DNAseq experiment was performed in *Populus deltoides* and *P. nigra*. Using CLC Bio Workbench 5.0, 10x Illumina reads of *P. deltoides* or *P. nigra* were assembled into contigs. All *P. deltoides* and *P. nigra* contigs were masked with the *P. trichocarpa* retrotransposon database. The pairwise comparisons indicated that the majority of *P. trichocarpa* retrotransposons occurred also in *P. deltoides* and *P. nigra*.

On the whole, the results support the view of retrotransposons as a community of different organisms in the genome, whose activity (both retrotransposition and DNA loss) has heavily impacted and probably continues to impact poplar genome structure and size.

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Poster Communication Abstract – 2A.48

DE NOVO ASSEMBLY AND GENOME STRUCTURE ANALYSIS IN THE *POPULUS* GENUS

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Next-generation sequencing technologies, genome assembly, Populus nigra, Populus trichocarpa

De novo sequencing of a genome is today accessible and affordable thanks to the advent of the next-generation sequencing technology that has made sequence data production accurate, cheap and fast. Since the release of this new technology, many genome sequences have been published but comparative and structural genomics analyses are still a challenging issue. Given the huge amount of sequence data available, nowadays a special effort has to be given to the latter analyses which are crucial to better understand both the evolution and the composition of the different genomes.

Exploiting the Illumina technology and a *de novo* assembly approach, the present work aims to obtain the genome sequence of an Italian genotype of *Populus nigra*, the native European poplar species which is very important for wood and paper industry. We sequenced the poplar tree at high coverage (90X) using different kinds of libraries in order to solve repetitions and allow the contig scaffolding: technical and critical aspects will be discussed. Then, we focused on two different softwares to perform the *de novo* assembly: performance comparisons and results will be provided. On the selected assembly (length 318 Mb and N50 4487 bp), we developed an analysis pipeline to characterize the contig content in terms of genes and repetitive elements, and sequence novelty compared to *P. trichocarpa*, the American poplar species sequenced using the Sanger method. A test experiment was run on almost 300 randomly-selected contigs in order to validate the pipeline which, due to the low error rate detected, proved to be efficient and accurate in comparing and characterizing the genome sequences concerned. We think our pipeline can be applied to the comparative genome analysis of different closely related organisms.

The comparative approach between the two *Populus* species will be exploited to introduce the concept of the pan-genome, which includes core genomic features common to both species and a dispensable genome composed of non-shared DNA elements that can be individual- or population-specific and important for explaining plasticity, phenotypic variation and heterotic behavior.

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Poster Communication Abstract – 2A.49

A CATALOG OF MOLECULAR DIVERSITY WITHIN *PRUNUS* GERMPLASM INFERRED FROM NEXT-GENERATION SEQUENCING DATA: BIOINFORMATIC APPROACHES AND CHALLENGES

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Next Generation Sequencing, Prunus persica, SNP, indel, bioinformatics

Genome analysis based on next generation sequencing (NGS) technologies provides a powerful approach for surveying molecular diversity among individuals, which in turn can generate tools for linkage mapping, association mapping, gene cloning, molecular breeding, population genetics, germplasm management, crop systematics and evolution.

'De novo' assembly of short reads is challenging as far as the size and complexity of genomes increases. A reference genome correctly assembled and annotated can help solving most of the problems, although several structural variants such as the movement of transposable elements, large insertions/deletions, segmental duplications and other genomic features are still challenging algorithms and automatic procedures.

We sequenced 16 Prunus accessions, that include 14 peach cultivars and species, one almond and one apricot varieties, using the NGS Illumina platform. We produced 64 to 109 bp long paired end reads from approx. 300-500 bp long fragments. The coverage varied from approx. 16 to 75 genome equivalents. Individual genomes were aligned using the doubled haploid peach cultivar 'Lovell' reference sequence recently released by the International Peach Genome Initiative (IPGI) (http://www.rosaceae.org/peach/genome).

In this work we present a catalog of molecular variants that can be mined with different bioinformatic approaches, namely SNPs (Single Nucleotide Polymorphisms), DIPs (Deletion/Insertion Polymorphisms), larger structural variations, which include movement of transposable elements, the so called copy-number variations, segmental duplications and other. Some of these variants, such as SNPs, are easily detected and many commercial and open-access software can perform the search. Others variants, such as the large structural variations, have still analytical approaches to be implemented or improved. For most variants, the possible methodological approaches are discussed and, when available, preliminary results are reported.

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Poster Communication Abstract – 2A.50

CORRELATION BETWEEN SINGLE NUCLEOTIDE POLYMORPHISM GENOTYPE AND PHENOTYPIC RESPONSE TO CADMIUM EXPOSURE IN *POPULUS* SPP.

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Salicaceae, phytoremediation, candidate genes, single nucleotide polymorphisms

Species within the genus *Populus* include potential phytoextractors of heavy metal ions from contaminated soils, and genetic markers predictive of performance would be a useful tool for selection and breeding. Here, we have identified sequence variation within seven target and three non-target genes among a set of 11 *Populus* spp. clones. Sequence variants were present in both the coding and non-coding regions; the former can potentially affect the functionality of the target genes. At the same time, the effect of exposure of the clones to cadmium ions on the morphology and the distribution of various metal ions was investigated by scanning electron microscopy microanalysis. A positive correlation was established between genetic variation, cadmium accumulation and its bioconcentration in the root.

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Poster Communication Abstract – 2A.51

HD-ZIP II TRANSCRIPTION FACTOR GENES CONTROL ADAXIAL-ABAXIAL PATTERNING IN *ARABIDOPSIS* LEAF MORPHOGENESIS

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Leaf development, Arabidopsis thaliana, HD-Zip

The Arabidopsis genome encodes for 10 Homeodomain-Leucine Zipper II (HD-Zip II) proteins. It has been previously shown that *ATHB2*, *HAT1*, *HAT2* (HD-Zip II γ subfamily), *HAT3* and *ATHB4* (δ subfamily) are induced by changes in the Red/Far Red ratio of the light environment. However, these genes are also tightly regulated during plant development with both distinct and overlapping patterns (Ciarbelli et al., Plant Mol. Biol. 2008, 68: 465-78). In order to understand the role of the light-regulated HD-Zip II genes in plant development, we have analyzed single and multiple mutants within γ and *δ* subfamilies. Here we present the phenotype of mutants in the *HAT3* and *ATHB4* genes. Young seedlings show a gradual loss of cotyledon and leaf expansion, up to completely radialized organs. The pattern of vascular development is also profoundly altered, in a manner that is tightly linked to lamina expansion. Fully radialized leaves lack procambial cells whereas trumpet shaped leaves show hyperproliferation of phloem with respect to xylem, a feature that is found in the vasculature of abaxialized leaves. *In situ* and GUS/GFP reporter analyses of the δ subfamily genes show that they are expressed in the adaxial side of cotyledons and leaves. Taken together, these data demonstrate that *HAT3* and *ATHB4* are required to specify adaxial identity in leaf morphogenesis. We are currently analyzing the molecular and genetic relationships between the d HD-Zip II genes and members of the HD-Zip III family genes, such as *PHB*, *PHV* and *REV*, key determinants of adaxial leaf identity.

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Poster Communication Abstract – 2A.52

EXPRESSION OF *TRICHODERMA HARZIANUMM* HYDROPHOBIN IN *SOLANUM LYCOPERSICUM*

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Biocontrol agent, Trichoderma-plant interaction, biotic stress tolerance

Trichoderma spp. are fungal antagonist, widely used as active ingredients in commercial bio-fungicides and bio-fertilizers. They have not only mycoparasitic activity, but they can also activate extensive metabolic changes in treated plants, resulting in a systemic resistance to a pathogen attack, thus indirectly altering plant-pathogen interactions. The mechanisms that regulate *Trichoderma*-plant-pathogen interaction have been deeply explored and many of the process key genes have been characterized. Genome sequence of several *Trichoderma* species have been completed, thus *Trichoderma* genus can be considered a very useful source of genes for biotechnological application as well as plant transformation.

Among molecules found essential for beneficial *Trichoderma*-plant interaction there are few hydrophobin and hydrofobin-like secreted proteins. One of these was a class II hydrophobin named HYTRA1. This has an antimicrobial activity and when applied to tomato plants is able to induce a defence response. In *in vitro* and *in vivo* assays, HYTRA1 directly inhibited pathogen development. It induced in tomato plants, depending upon the concentration, a multiplicity of effects, in fact it activated oxidative burst, the antioxidant system, and ISR with the accumulation of defence-related compounds important in plant defence.

To study the role of hydrophobin in *Trichoderma*-plant interaction, *Solanum lycopersicum* was transformed with *Hytra1* gene of *T. harzianum*, fused to a sequence encoding for a myc peptide.

Transformed plants were confirmed by PCR and RT-PCR. A phenotypic analysis revealed differences between transformed plant and control in terms of plant morphology and size. The performance of transgenic plants when challenged with different biotic stressors is presented and discussed.

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Poster Communication Abstract – 2A.53

TOWARDS PHENOMICS OF THE SEQUENCED GENOMES OF THE CULTIVATED TOMATO AND ITS WILD ANCESTOR *SOLANUM PIMPINELLIFOLIUM*

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Tomato wild species, BILs, QTL, PCR-based markers

The genomes of tomato (*Solanum lycopersicum*) and its wild progenitor, *Solanum pimpinellifolium,* have been sequenced (http://solgenomics.net/). In contrast to most wild tomato species, which produce green inedible fruits, *Solanum pimpinellifolium* yields small (~1 gram) brightly colored red fruits commonly known as "currant" tomatoes. Until now *Solanum pimpinellifolium* remained largely inaccessible to high-resolution genetic dissection due to its low level of marker polymorphism. However, the recent sequencing efforts revealed multiple SNPs that could be used for the mapping as well as for the identification of the functional SNPs that differentiate the cultivated tomato from its wild progenitor. Our objective was to re-visit the phenotypic diversity in a Backcross Inbred Line (BIL) population (178 lines) derived from a *Solanum lycopersicum* (cv. E6203) x *Solanum pimpinellifolium* LA1589 cross. This will create an extremely valuable data resource for directly associating SNPs with domestication phenotypes. Towards this aim we have anchored the BIL population to a framework of PCR-based markers covering the entire genome, and we are now in the process of phenotypic evaluation of the BIL population for numerous traits of interest. By combining the large phenotypic databases with the *Solanum pimpenellifolium* genome sequence that is now available we aim to shed light on numerous QTL that underlie phenotypic variation in different biological areas.

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Poster Communication Abstract – 2A.54

TOMATO R-GENES GENOME-WIDE SORTING

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R-protein, Lycopersicon esculentum genome, gene cluster, domain

A complete set of candidate disease resistance (R) genes was identified in the genome sequence of tomato (*Lycopersicon esculentum* var. Hein 1706). These putative R genes were characterized with respect to structural diversity, phylogenetic relationships and chromosomal distribution, and compared with R genes that have now been cloned from different monocot and dicot plant species. We found more than 700 coding sequences, including TNL proteins, CNL proteins, RLP proteins RLK proteins, as well as, other different domains arrangements. Genes were not uniformly distributed on chromosomes. Many of them remain in clusters, containing highly similar gene members. Fifty-four per cent of predicted R-genes were located in 121 gene clusters, and 81 of these gene clusters showed extensively gene duplications. The structural and genetic diversity that exists among R-proteins in tomato is remarkable and suggests that diversifying selection has played an important role in the evolution of R genes in this agronomically important species.

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Poster Communication Abstract – 2A.55

MOLECULAR CHARACTERIZATION OF GENETIC DIVERSITY IN SYMPATRIC WHITE OAK SPECIES (*QUERCUS* SPP.)

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Gene flow, Quercus frainetto, Q. petraea, Q. pubescens, EST-SSRs

Gene flow in genus *Quercus* has been intensively studied because of the characteristic of oak species to intercross and hybridization is a common phenomenon that frequently co-occurs in mixed stands.

The mean aim of the study is to evaluate the rate of gene flow and to analyze genetic diversity in molecular traits within a woody community of three sympatric and closely related white oak species of *subgenus Quercus* (*Quercus frainetto* Ten., *Q. petraea* Liebl. Matt. e *Q. pubescens* Willd.), using nuclear microsatellites (EST-SSRs).

A total of 268 oak trees, from 9 stands located in a natural mixed oak forest in central Italy, were genotyped at 12 microsatellite markers, using a PCR 12-plex protocol (Guichoux *et al*, 2011).

This study reports the results of genetic differentiation among the three species in particular to characterize the rate of hybridization and introgression and evaluate gene flow among the three species. All nuclear markers resulted 100% polymorphic and the number of alleles varies from 3 alleles in *Q. frainetto* to 14 in *Q. petraea* with an average of 8.611 alleles per locus.

The Bayesian cluster analysis (without *a priori* information), demonstrated elevated capacity in identifying genetically pure and mixed group of individuals while revealing inter-specific gene flow. Each of the groups individuated is characterized by a set of allele frequencies per locus which confirms the morphological traits (Viscosi & Fortini, 2011) recognizable as three species *Q. frainetto, Q. petraea* and *Q. Pubescens*. The individuals were not correctly assigned only in 0.75% of total samples. The overall percentage of hybrids varies considerably in the area of study ranging from 3.33% to 36.67%

In addition, the genetic composition of stands gave proof of evidence of relationships between environmental factors and spatial distributions of pure species and their hybrids.

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Poster Communication Abstract – 2A.56

GENETIC CHARACTERIZATION OF KNOWN AND NOVEL COMPONENTS OF PIRNA PATHWAYS IN *DROSOPHILA MELANOGASTER* GONADS

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piRNAs pathways, Drosophila melanogaster, crystal-Stellate, transposons, cryptic variation

In the germ tissues, the RNA interference pathway provides a crucial defence mechanism against transposons, which are primarily transmitted by inheritance. In Drosophila, the specialized Piwi-interacting RNA (piRNA) pathways repress transposons and other repetitive sequences in gonads. piRNAs are generated by a Dicer-independent mechanism and function through the Piwi, rather than the Ago, Argonaute subfamily proteins. Flies have three closely related Piwi proteins, Piwi, Aubergine and Ago3, which use piRNAs to silence transposons and other parasitic DNAs in gonads (Ghildiyal and Zamore, 2009). In ovaries, distinct piRNA pathways with differing components function in ovarian germ and somatic cells (Li et al., 2009; Malone et al., 2009). The two piRNAs pathways produce piRNAs with two molecular mechanism: the primary piRNA pathway, Piwi-dependent, acting in the somatic cells, the so called ping-pong pathway, Aub- Ago3-dependent, acting in the germline (Sentia and Brennecke, 2010).

In testes, the piRNAs production remains largely unknown. In fly testes the most abundant piRNAs (~70%) associated with Aubergine corresponding to *crystal* and *Stellate* sequences (Nagao et al., 2010). The *crystal-Stellate* system is one of the most studied examples of heterochromatin-euchromatin interaction (Bozzetti et al., 1995). The modifiers of the *crystal-Stellate* system were demonstrated to be key genes implicated in the piRNA pathway, as *aubergine, spindle E, hsp83* (Klattenhoff and Theurkauf, 2008; Specchia et al., 2008; Specchia et al., 2010).

Here we show the data on the behavior of some *crystal-Stellate* modifiers, in particular *hsp83* and the Drosophila Fragile X homolog gene, *dFmr1*, in the silencing of somatic or germline specific transposable elements and *Stellate*-like sequences. This study contributes to shed light on the mechanisms of the piRNAs production and function in *Drosophila melanogaster* gonads.

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Poster Communication Abstract – 2A.57

IDENTIFICATION AND EXPRESSION ANALYSIS OF CLASS C AND D MADS-BOX GENES IN *ORCHIS ITALICA* (ORCHIDACEAE)

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AGAMOUS-like gene, SEEDSTIK-like gene, flower development, Orchidaceae, Real Time RT-PCR

Two MADS-box cDNA, called *OitaAG* and *OitaSTK*, respectively, were identified in inflorescence tissue of the *Orchis italica* (Orchidaceae) during a study of genes involved in flower development in orchids. Based on nucleotide and deduced amino acid sequence analysis, *OitaAG* resulted homolog of the class C MADS-box gene *AGAMOUS* and *OitaSTK* homolog of the class D MADS-box gene *SEEDSTICK* of *Arabidopsis thaliana*, where class C genes are involved in petal and stamen development and class D genes drive the ovary formation. The orchid flower includes an external whorl of three sepaloid petals (outer tepals), two lateral inner tepals, a median inner tepal (lip) and the reproductive whorl in which male and female reproductive tissue are fused (column). Pollinia and ovary are located at the top and at the base of the column, respectively. In orchids, the ovary development is triggered by pollination.

In order to analyze the quantitative relative expression pattern of the *OitaAG* and *OitaSTK* genes during the development of the flower tissues, Real Time RT-PCR experiments were conducted on the different floral tissues of *O. italica* dissected from immature and mature inflorescence. In addition, the expression of both genes was checked in immature and mature ovary, before and after manual pollination. The quantitative relative expression of the *OitaAG* and *OitaSTK* genes in the different tissues were calculated applying the $\Delta\Delta C_T$ approach, using the actin *OitaAct* as the endogenous control gene and leaf cDNA as the reference sample.

In the immature inflorescence, *OitaAG* is expressed in outer and inner tepals and only a weak expression is detectable in lip and column. In the mature inflorescence, inner tepals and lip show a significant increment of the *OitaAG* expression, whereas its levels in outer tepals and column remain approximately constant. In addition, a high amount of *OitaAG* mRNA is detectable in mature ovary before pollination, whereas after pollination its expression slowly decreases.

The *OitaSTK* gene is expressed in outer and inner tepals and in lip dissected from immature inflorescence; a strong increment of its expression is detectable in lip tissue of the mature inflorescence. The highest amount of *OitaSTK* mRNA is detectable in the mature ovary before pollination. After pollination, the expression of *OitaSTK* is still high, even though less abundant when compared to the mature ovary before pollination.

The expression patterns of the *OitaAG* and *OitaSTK* genes of *O. italica* do not fully overlap to those of the class C and D MADS-box genes, respectively, described in *Arabidopsis* and other model organisms, leading to hypothesize possible different roles of these genes during the development of the orchid flower.

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Poster Communication Abstract – 2A.58

IDENTIFICATION AND VALIDATION OF THE RESPONSE ELEMENTS FOR THE P53 FAMILY MEMBERS IN THE GENE ENCODING THE MITOCHONDRIAL TUMOR SUPPRESSOR PROLINE DEHYDROGENASE

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Proline dehydrogenase/proline oxidase, responsive elements, p53 family, apoptosis, metabolism

p53 regulates cell cycle arrest, apoptosis, autophagy and other processes in response to various stresses. A recent work has discovered additional functions for this tumor suppressor gene, i.e. the control of cellular metabolism and energy production. p53 was previously shown to markedly upregulate the metabolic enzyme Proline dehydrogenase (PRODH), which catalyzes the first step in proline degradation and contributes to p53 function inducing ROS mediated apoptosis in response to genotoxic stress. Characterization of the p53-REs in PRODH, as well as determination of the inducibility by the other p53 family members is critical to understanding its regulation in normal or pathological conditions.

Induction of endogenous PRODH in response to genotoxic damage or p53 family members overexpression was observed in mammalian cell lines. Bioinformatic analysis identified eight putative p53 consensus sequences in the *PRODH* gene, located both in the promoter and intronic regions. We selected five REs whose sequences showed higher affinity for p53 and correspondent yeast reporter strains were created using the "*delitto perfetto*" approach. A well established yeast transactivation assay was applied to analyze the induction of the reporter gene luciferase through the specific PRODH p53RE upon modulated expression of p53, p63 and p73 proteins. Furthermore, chromatin immunoprecipitation (ChIP) assays were carried out in human tumor cell lines.

Three of the p53-REs responded to p53 and to the other members of the family, although at different extent. These results will help to elucidate fine regulation of PRODH, a protein involved in many metabolic pathways often deregulated in cancer.

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Poster Communication Abstract – 2A.59

GENE EXPRESSION PROFILING AROUND THE CLOCK IN THE ANTARCTIC KRILL (*EUPHAUSIA SUPERBA*)

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Antarctic krill, circadian clock, normalized library, next generation sequencing, microarray

The Antarctic krill (*Euphausia superba*) is a key species of the Atlantic sector of the Southern Ocean ecosystem and plays an important role both as feeder of algae, bacteria and micro-zooplancton and as a prey of vertebrates. It displays a large daily vertical migration that makes a significant amount of biomass available as food for predators near the surface at night and in deeper waters during the day. Despite the great interest on this species, however, the molecular and physiological mechanisms that determine its abundance and distribution are still very poorly understood. The genome sequence of krill is not yet available and therefore the systematic sequencing of cDNA libraries [1] represents a powerful approach to identify large numbers of transcripts that could be used in gene expression and functional genomics studies. To this purpose we produced and pyrosequenced a novel normalized cDNA library characterized by two steps: the "whole transcriptome amplification (WTA)" and "Duplex-specific nuclease (DSN) normalization". This strategy allowed to optimize the discovery rate of the random sequencing process by equilibrating the final representation of abundant and rare transcripts. To increase the probability of identifying circadian clock genes we constructed a normalized library from krill sampled at different times of the day over a complete 24-hour cycle. Using the 454 Titanium technology we have identified 89,230 high-quality reads which were assembled into 350 overlapping clusters and 10,647 singletons (>200 nt) resulting in a total of 10,987 putative transcripts. Sequences generated by our group and all available *E. superba* sequences from public databases, at present 6,142 ESTs [1, 2] and 777,544 [3] 454 reads (Taxonomy Browser at NCBI, June 2011), have been assembled to create the first krill microarray platform, named Krill 1.2, with a total of 32,217 different probes. We produced 8x60K microarrays (Agilent Technologies) which allowed the analysis of eight different samples on single slides. Using krill 1.2 platform we defined gene expression signatures of specimens collected in the Ross Sea at five different time of the day, during the Antarctic summer, over a complete 24-hour cycle in order to characterize the krill circadian transcriptome [4]. Our work gives a first insight into the molecular mechanisms that allow krill's clock to interpret environmental signals and modulate physiology and behavior accordingly.

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Poster Communication Abstract – 2A.60

A FIRST SURVEY OF THE GENOMIC ORGANIZATION OF THE T-CELL RECEPTOR GAMMA LOCUS IN *TURSIOPS TRUNCATUS*

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T-cell receptor, genome, genomic organization, TRG locus, Cetartiodactyla

The dolphin is one of the species whose genome is being sequenced as part of a comparative genomic analys aimed to identify functional elements conserved across mammals.

The phylogenetic position of the bottlenose dolphin within the mammals is still uncertain, but recent molecular morphological and paleontological studies suggest that Cetacea share a close relationship with Artiodactyla, so it has been proposed that these two orders should be combined into a single new order, the Cetartiodactyla.

Artiodactyls (i.e. ruminants and pigs) occupy a particular immunological niche with regard to the cell-mediated immune response. They are defined "γδ high species", referring to the high level (20 to 50%) of γδ T cells present in blood rather than in epithelial cell-rich tissues. On the other hand, species such as human and mouse are "γδ low species" because only 5% of their peripheral T-cell pool is composed of γδ T cells.

The wide and diversified repertoire of the γδ chains certainly depends by the genomic organization of γ and δ loci.

In particular, the TRG locus of sheep and cattle, was apparently originated by reiterated duplication of functional gene cassettes, and is split into two cassette arrays localized in different chromosomal regions.

Here, we report data about the genomic TRG locus organization in dolphin.

The comparison of the sequence retrieved from HGSG Genome Data with human and cattle has resulted in two adjacent but non-contiguous contigs (96017 bp and 91079bp). The 5' and 3' boundaries of the locus are defined by the AMPHU and STARD3 genes and its putative length is at least 138 kb. Only two variable genes (TRGV) orthologous to human V9 and V11 are present in the first contig while three joining (TRGJ) and one constant (TRGC) genes orthologous to the sheep cassette C5, were found in the second contig.

Genome walking and Long PCR experiments indicate that the dolphin TRG locus is organized in a single V-J-C cassette. This gene organization is reminiscent of cattle and sheep, whose TRG loci consist, at each chromosomal region, of four or three tandemly repeated V-J-C cassettes, arranged in the same transcriptional orientation. The main difference between Bovidae and Cetacea we observed is the very low number of TRG genes found in dolphin.

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Poster Communication Abstract – 2A.61

HORSE MITOCHONDRIAL GENOME ANALYSES TO DEFINE THE ORIGIN OF ANCIENT HUMAN POPULATIONS

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Maremmano breed, mitochondrial genome, phylogenetic analysis

Since the use of horses was profoundly connected to human activities in both pre-historical and historical times, detailed molecular and phylogenetic analyses of equine mtDNA can increase our knowledge on both horse domestication and ancient human populations. In this species, genetic association studies are also favoured by the availability of studbooks with pedigrees and racing records. Maremmano's studbook could be traced back to 4 stallions and 440 mares. The Maremmano is an Italian warmblood horse mostly bred in the provinces of Grosseto and Viterbo (Central Italy). It is believed that this breed originated in Tuscany from ancient local populations living along the Tyrrhenian coast during the Etruscan time and was influenced by ages of crossing with other horse breeds. In order to investigate this theory from a genetic point of view, following the approach recently used to analyze humans (modern Tuscans) and bovines (Maremmana and Chianina breeds), we evaluated the mitochondrial DNA variation of 73 unrelated horses pre-selected on the bases of an accurate genealogical analysis of the maternal lines. Control region sequencing (nps 15491-16100) revealed 47 different haplotypes, ascribable into 12 major haplogroups. Particular interest was arisen from samples belonging to A4, (relatively abundant with 11.0 % of frequency) because this haplogroup clearly shows a frequency peak in the Near East. On the basis of these preliminary data, in order to avoid redundancies and to achieve a good representation of the entire equine "natural" mtDNA variation, we selected 20 samples for complete sequencing of the entire mitochondrial genomes. Excluding ambiguous sites and the 16129-16360 short tandem repeat, 20 different haplotypes were identified in the sequences and evolutionary history was inferred by a parsimony approach.

The resulting data show a peculiar phylogenetic connection between the Maremmano horse and some typical eastern breeds.

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Poster Communication Abstract – 2A.62

THE REPERTOIRE OF GAMMA/DELTA TCR IN DROMEDARY IS DIVERSIFIED BY SOMATIC MUTATION AND CDR3 DIVERSIFICATION

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Camelus dromedarius, TRG and TRD loci, somatic mutation, protein modeling

In Jawed vertebrates the T-cell immune response is mediated by the T-cell receptor ab or gd heterodimers, encoded by complex multigene families. The genetic information for these receptors is carried by a germline pool of variable (V), joining (J), diversity (D), and constant (C) genes that undergo somatic DNA recombination to generate receptors with diverse binding specificity. It has been suggested that the gamma/delta T cells represent a bridge between the innate and the adaptive immune systems. They show in fact unique features if compared with the more abundant alpha/beta T cells, e.g. a preferential distribution in epithelial and mucosal sites, and, in addition to the MHC-restricted one, an Ig-like antigen recognition mechanism. Diversity in receptor structure and mechanism of diversification is now being discovered in a lineage- and taxon-dependent manner, supporting the idea that metazoan immunity is an evolutionarily plastic system. Camelidae occupy a peculiar immunological niche within mammalians, since, in addition to conventional antibodies, the serum of these animals contains a significant amount of antibodies composed solely of paired H-chains (HCAbs). This feature, together with the phylogenetic placement of Camelidae among Cetartiodactyls, makes the study of the dromedary cellular immunity very intriguing.

We investigated T cell-mediated immunity in Camelidae, focusing on the TR delta (TRD) and TR gamma (TRG) repertoire in dromedary spleen. By a combination of 5'RACE and RT-PCR experiments, we first identified three TRDV subgroups and five joining (TRDJ) genes, and then two TRGV subgroups and two TRGJ genes. We provide evidence that the high diversity in sequence and length of the third complementarity determining region (CDR3) is a major component of TR delta and gamma chain variability. Moreover, comparison with the corresponding germline genes allowed us to show, for the first time in a mammalian organism, that productively rearranged TRDV and TRGV genes can undergo somatic mutation: the mutation rate per base pair is 0.013 (TRDV4 region) and 0.008 (TRGV1 and TRGV2 regions). A computational approach has been applied to determine the protein structure of the variable region of γδ heterodimers. The complex TRGV1-TRDV4 and TRGV2-TRDV4 were modeled on the human counterpart γδ TCR (PDB code: 3OMZ). From these structures it appears that solvent accessible surface area is higher in TRVG1 than in TRVG2 and this is due to different steric hindrance of the side chains mainly localized in the CDR region. It is possible that amino acid variations in gamma/delta T cells, which respond to antigens independently of antigen processing and MHC presentation, may be more easily

tolerated and maintained during evolution. This insight could be significant for understanding the evolution of the mechanisms generating diversity in the vertebrate immune system.

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Poster Communication Abstract – 2A.63

THE STABILITY OF INSTABILITY: OCCURRENCE OF A FUNCTIONAL TELOMERASE IN THE HOLOCENTRIC CHROMOSOMES OF APHIDS

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Aphid, holocentric chromosomes, telomere, telomerase, intraindividual chromosomal instability

The structure of the telomeres of four aphid species (*Acyrthosiphon pisum*, *Megoura viciae*, *Myzus persicae* and *Rhopalosiphum padi*), evaluated by Southern blotting and fluorescent *in situ* hybridization, revealed that each chromosomal end consists of the (TTAGG)n repeat. The presence and the sequence composition of a telomerase coding gene has been verified in *A. pisum* genome, revealing that aphid telomerase presents about 20% sequence identity with the invertebrate and vertebrate homologues and possesses the two main domains involved in telomerase activity thus demonstrating that it is a functional enzyme. Interestingly, telomerase expression has been observed in different somatic tissues suggesting that in aphids the telomerase activity could be not restricted as in human cells. The study of telomeres in a *M. persicae* clone with chromosome number ranging from 12 to 17 even within each embryo, evidenced that aphid telomerase can initiate the *de novo* synthesis of telomere sequences at internal breakpoints resulting in the stabilization of the chromosomal fragments. Chromosome instability is a peculiar feature of malignant cells, whereas it is a very rare phenomenon in physiological situations and even more in whole organisms. The observed rearrangements involved prevalently X chromosome, but also autosomes 1 and 3. Literature data revealed that autosomes 3 and, more rarely, 1 are the chromosomes mostly involved in *M. persicae* karyotype variations. On the contrary fragmentations occurring at the X chromosomes are absolutely rare in aphids not only in natural populations, but also in X-ray irradiated specimens.

The random-breakage model of chromosome evolution has been the dominant paradigm for several years. However, further analyses suggested that recurrent breaks are found in fragile regions or hotspots so that the random breakage model required substantial reassessment in favour of models that put the architecture of the chromosomes in a pivotal position for revealing the molecular basis of chromosomal evolution among species. Several comparative mapping studies in a wide variety of closely related eukaryotes showed a relationship between large-scale chromosomal rearrangement and repetitive DNA. The nature of the repetitive DNA within these breakpoint regions varies significantly, from clusters of rRNA and satellite DNAs to various mobile elements. The *M. persicae* clone analysed showed chromosome fragmentations generally located within satellite DNAs clusters, which seem therefore to represent fragile sites that could be at the basis of the intraindividual chromosome fissions.

Experiments of male induction assessed that this *M. persicae* clone is an obligate parthenogenetic population. The holocentric nature of aphid chromosome could justify karyotype variations, whereas the reproduction by apomictic parthenogenesis, together with a higher

telomerase expression level, favoured the stabilization of the observed intraindividual chromosome instability.

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Poster Communication Abstract – 2A.64

IDENTIFICATION OF NOVEL LTR-RETROTRANSPOSONS IN THE GENOME OF *CULEX QUINQUEFASCIATUS*

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Culex quinquefasciatus, LTR retrotransposons, LTR_STRUC

Transposable elements are ubiquitous component of eukaryotic genomes and, besides their mutagenic role, they are considered as the major source of variability that can change genomes and their expression, either considering short term or large evolutionary scale time. The post-genomic era offers a great opportunity to shed light on the evolution of mobile genetic elements with respect to eukaryotic genome. In the last years a special interest in the field of mosquitoes' genomics is highlighted by the completion of three genomic sequences (i.e. *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus*); this interest come from their role to function as vectors of virus-borne diseases. Few transposon families have been described in the Culex genus before the sequencing of *C. quinquefasciatus* genome. The genomic sequence analysis recently performed by Arensburger et al. (1) has revealed that nearly 30% of this genome is composed of TEs. The TE-related sequences described in Arensburger et al. were deposited in the *TEfam* database.

The genome of *C. quinquefasciatus* has been analyzed using the LTR_STRUC program. Thirty novel families of LTR retrotransposons have been identified. Furthermore a group of non-autonomous elements has been identified, featured by tandem repeated sequences between the LTRs and apparently unrelated to any known Culex retrotransposon family.

The potential role of the LTR-retrotransposon insertions on the host gene structure has been studied, and several insertions that may potentially contribute to the mature transcripts of endogenous genes have been identified.

These results integrate the existing data on the genomics of an important disease vector.

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Poster Communication Abstract – 2A.65

EVOLUTIONARY CHROMOSOMAL REARRANGEMENTS LEAVE UNCHANGED INTRANUCLEAR POSITION OF TRANSLOCATED LOCI IN PRIMATE CELLS

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Human genome, interphase nuclei, in situ hybridization, BAC probes, chromosome evolution

Intranuclear spatial repositioning of genes has been described in cell differentiation and in some genetic diseases, showing a correlation between radial nuclear location (RNL) of genes and expression profile. It should be hypothesized that orthologous genes have similar RNL in cell nuclei from different species to preserve specific expression pattern. Thus, to evaluate spatial chromatin organization and the related genomic features in different lineages, we analysed RNL of eighteen different loci, involved in evolutionary translocation/inversion events, in cell nuclei from human, gorilla and macaque. Our analyses were performed by 2-D in situ hybridization with human BAC probes, and results showed a general conserved RNL of the investigated loci. This indicates that chromosomal rearrangements fixed during primate chromosomal evolution seem to leave unchanged the RNL of the involved loci, in order to preserve original transcriptional activity related to the location of genes in specific subnuclear contexts.

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Poster Communication Abstract – 2A.66

MOLECULAR CHARACTERIZATION OF *TUBER MELANOSPORUM* RESPONSES TO LIGHT

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Light responses, hypogeous organism, chimeric photoreceptors, Neurospora transformants

The first genomic sequence of a *Tuber* spp (*T. melanosporum*) has been published in 2010 (Martin et al, Nature 464,1033-1038). The presence among the annotated sequences of ORFs corresponding to known photoreceptors confirmed the presence of light induced phenomena previously observed only in a different Tuber specie (*T borchii*). In fact we already pubblished data on the existence of light dependent responses in this organism with an underground life cycle.

We identified in *T. melanosporum* different classes of light photoreceptors: as phototropins (WC-1WC-2), opsins, phytochome and velvet like transduction complex. The most conserved system is that of White collar proteins, but also the velvet complex appears conserved, while the retinal less opsine and the heavily rearranged phytochrome are more enigmatic. We have data from biochip that reveals that all the putative photoreceptors are expressed in tuber and in particular each of them is mainly expressed in a specialized structures of Tuber as fruitbody, mycelium or mycorrhiza.

Special attention we put on the characterization of the sensor domains.of the photoreceptors. In particular we would like to measure the minimal amount of photones necessary to activate an hypogeous sensor compared with the light required to activate a Neurospora LOV domain .

We constructed recombinant WC-1 molecules containing the original Neurospora LOV domain or the LOv domain from *T. melanosporum* or *borchii* and we used them for Neurospora transformation. We obtained new strains that have been characterized by Southern analysis and PCR. We are now characterizing the peculiar properties of Tuber LOV domain in responding to light activation: Kinetic of light dependent mRNA, time of photoreceptor recovery and others.

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Poster Communication Abstract – 2A.67

MOBILITY OF TTD1A RETROTRANSPOSON BY STRESS MODULATE RESISTANCE EXPRESSION GENE IN DURUM

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Salt stress, light stress, Triticum durum, retrotrasposon

Long terminal repeat retrotransposons are the most abundant mobile elements in the plant genome (Flavel et al. 1992). Stress modulation of retrotransposons may play a role in generating host genetic plasticity in response to environmental stress. Their success depends on the ability of their promoters to respond to different signaling pathways that regulate plant adaptation to biotic and abiotic stresses. In fact, their promoter elements are similar to those of plant defence genes and may bind similar defense-induced transcription factors. In this work, we have isolated a new Ty1-copia-like retrotransposon, named Ttd1a from the *Triticum durum* L. genome. To get insight into stress activation pathways in Ttd1a, we investigated the effect of salt and light stresses by RT-PCR and S-SAP profiling (Woodrow et al. 2010). We screened for Ttd1a insertion polymorphisms in plants grown under stress and showed that one new insertion was located near the resistance gene. Our analysis showed that the activation and mobilization of Ttd1a was controlled by salt and light stresses, which strengthened the hypothesis that stress mobilization of this element might play a role in the defence response to environmental stresses. Finally, using a retard mobility assay in *Triticum durum* L. crude extracts, we showed that the CAAT motif present in the Ttd1a retrotransposon promoter, is involved in DNA–protein binding under salt and light stresses and therefore in the regulation of Ttd1a activity (Woodrow et al. 2011). Data presented in this paper suggest that nuclear proteins can interact with the CAAT motif either directly or indirectly and enhance Ttd1a by a specific ligand-dependent activation under stress.

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Poster Communication Abstract – 2A.68

STUDY OF A MAIZE VIVIPAROUS MUTANT IMPAIRED IN THE LAST STEP OF ABA BIOSYNTHESIS AND IN THE Moco PATHWAY

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ABA, Moco, viviparous

*vp*404* is a viviparous mutant with light green seedlings, reduced chlorophylls and carotenoids content and lower ABA level in both embryo and seedling tissues when compared to wild-type.

Because of the analogies between the phenotype of this mutant and the one of *vp10* and *vp15*, impaired in the Molybdenum Cofactor (Moco) pathway, we crossed the *vp*404* mutant with TB-10L and 5L, uncovering *vp10* and *vp15* respectively. We also performed a complementation test with all viviparous mutants with green seedlings reported in the literature.

The result of both complementation and TB-A tests suggest that *vp*404* defines a new *vp* gene whose product is presumably involved in Moco biosynthesis. Candidate genes are *Zmcnx* genes encoding CNX proteins involved in Moco-O biosynthesis and the gene encoding Moco Sulfurase that transforms Moco-O to Moco-S.

Moco-O is required for the activity of both Nitrate Reductase (NR) and Sulphite Oxidase (SO), whereas moco-s is required for the activity of Abscisic Aldehyde Oxidase (AAO), involved in the last step of ABA biosynthesis, as well as for Xanthine Dehydrogenase (XDH) activity.

To verify the possibility that *vp*404* is a mutation of one of the candidate genes, we compared SO as well as AAO, and XDH enzyme activity, in wild-type and mutant embryo tissues.

In the mutant, analysis of AAO (directly involved in ABA biosynthesis) and XDH, both requiring Moco-S, shows that their activity is almost undetectable when compared to the one of wild type tissues. On the other hand the SO enzyme, requiring Moco-O, shows significant activity even in the mutant.

These results are expected if *vp*404* is impaired in the Moco pathway, in addition the high SO activity in the mutant points to a block in the step where Moco Sulfurase transforms Moco-O in Moco-S. To verify this hypothesis we are performing further molecular and genetic analysis.

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Poster Communication Abstract – 2A.69

WHEAT GENOTYPING BY ILLUMINA GOLDENGATE ASSAY: A TEST CASE

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SNP, Illumina GoldenGate assay, genotyping, wheat

Single nucleotide polymorphisms (SNPs) are efficiently used for building high resolution genetic maps, studying population evolutionary history and performing genome-wide association mapping experiments. These applications usually require genotyping of thousands of SNPs in a large number of individuals. Although a number of SNP genotyping assays are available, most of them are designed for SNP genotyping in diploid individuals. Genotyping of polyploid organisms is naturally complicated by the presence in the nucleus of homeologous and paralogous genes. In fact, SNP scoring in polyploid species might result in more than 3 clusters (AA, BB, AB) making SNP clustering sometimes more difficult and less accurate.

In this work, we used the Illumina GoldenGate assay for the medium-high throughput SNP genotyping of tetraploid and hexaploid wheat accessions. This technical approach allows to carry out the assay reactions directly on genomic DNA without a preliminary PCR amplification. Afterwards, the allelic state at an SNP locus is obtained using a custom oligo pool (OPA). These oligos are designed on the non-varying sequences around each SNP for 48 up to 384 SNP simultaneously, thus resulting cost, time and work effective and easily scaling up.

In particular, we used a 384-plex OPA (169 A-genome, 166 B-genome and 49 D-genome SNPs) for the screening of 420 polyploid wheat accessions including 210 lines of *T. durum*, 94 lines of *T. aestivum*, 116 lines of *T. dicoccoides*, and also 25 emmer lines (*T. dicoccum*) and three diploid species related to wheat, *T. urartu* (A genome), *A. speltoides* (B genome) and *A. tauschii* (D genome). Clustering of Cy3 and Cy5 normalized intensities in a Cartesian plot was used to infer the SNP genotypes.

The evaluation of these 384 SNPs allowed us to identify an initial SNP core-set that includes 191 SNPs generally scorable in any wheat material. Moreover, evaluating separately the different wheat sub-groups, the useful SNP number changed, as expected, in relation with the homology and genome polyploidy analyzed.

This study reinforces the idea that the GoldenGate assay could be a very efficient tool for high-throughput genotyping of polyploid wheat, opening new possibilities for the analysis of genetic variation in wheat and dissection of genetic basis of complex traits using association mapping approach.

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Poster Communication Abstract – 2A.70

DEEP SEQUENCING-BASED CHARACTERIZATION OF WHEAT miRNome UNDER STRESS CONDITIONS

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Wheat, miRNome, abiotic stress

The molecular response of plants to stress is a complex process based both on the modulation of transcriptional activity of several stress-related genes and on post-transcriptional regulation. MicroRNAs are endogenuos small non-coding RNAs of about 20-24 nt that are known to play key regulatory roles in plant response to stress, besides being involved in development and morphogenesis. Most of the reports that link a specific microRNA to stress response are based on its down/up-regulation when plants are exposed to stress. Usually stress-induced miRNAs target negative regulators of the stress responsive genes, while the targets of stress down-regulated microRNAs are positive regulators or stress up-regulated genes.

In this work the wheat miRNome in drought-stressed plants has been characterized by a deep sequencing approach on Illumina GAIIX and candidate stress-induced microRNAs have been identified. Besides, an *in silico* analysis of gene expression for the identification of putative stress-related microRNAs has been developed.

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Poster Communication Abstract – 2A.71

EVALUATION OF THE SYNTENY FOR 5AS CHROMOSOME IN *TRITICUM* SPECIES WITH DIFFERENT PLOIDY LEVELS

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Synteny, wheat, genetic maps, evolution

In the frame of the project "Physical mapping of wheat chromosome 5A", we have undertaken an investigation about the synteny level of the short arm of chromosome 5A (5AS) among different species of *Triticum* genus characterized by a different ploidy level and evolutionarily separated on a time scale in order to get insights into possible chromosomal rearrangements occurred during evolution. The analyzed species were *Triticum aestivum* (AABBDD; 2n=42), *Triticum durum* (AABB; 2n=28) and *Triticum monococcum* (AA; 2n=14). In details, we relied on four mapping populations: [1] Chinese Spring (CS, *T.aestivum*) x Renan (*T.aestivum*); [2] CS x CS disomic substitution line for chr. 5A (*T. turgidum ssp dicoccoides)*; [3] Latino (*T. turgidum ssp durum*) x MG5323 (*T. turgidum ssp dicoccum*); [4] DV92 (*T. monococcum*) x G3116 (*T. monococcum*). High density genetic maps have been developed for the short arm of wheat chromosome 5A in these four populations using SSR (simple sequence repeat), SSR-EST (SSR-expressed sequence tags), TE junction (trasponable elements) and COS (conserved ortholog set) comparative anchor markers. The specificity of these markers for chromosome 5AS has been assayed using nulli-tetrasomic lines derived from the reference cultivar Chinese Spring. Moreover the physical position of the developed markers has been assigned to deletion bins of 5AS through the utilization of deletion lines. The evaluation of syntenic blocks and non-conserved regions among the homologous segments of different *Triticum* species is reported, while the mapping of EST-based markers allowed identification of syntenic regions in the rice and brachypodium genomes. Identification of possible rearrangements in the different 5AS genetic maps of wheat provide valuable information about the subsequent steps on the BAC contigs anchoring while the consensus map deriving from the integration of these four maps will provide a fundamental tool to link the genetic and physical maps.

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Poster Communication Abstract – 2A.72

TRANSCRIPTOMIC ANALYSIS OF DROUGHT AND HEAT RESPONSES IN DURUM WHEAT AND eQTLs MAPPING TO IDENTIFY THE LOCI CONTROLLING THE MOLECULAR RESPONSE TO DROUGHT

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Triticum durum, drought, heat stress, expression analysis, expression-QTL mapping

The durum wheat cultivars "Cappelli" and "Ofanto" are characterized by contrasting behavior in response to high temperature and drought stress.

The transcriptome profiles of the two cultivars flag leaves subjected to water stress, heat stress and a combined stress at booting stage were compared based on Affymetrix microarray analysis. The analysis has identified 1850 stress responsive probe sets characterized by a significantly different stress expression profiles in Cappelli vs Ofanto. These genes are almost not differentially expressed in response to drought both in Cappelli and Ofanto. On the contrary, Cappelli showed a more pronounced molecular response to heat stress. Finally these genes showed similar expression levels in response to the combined stress.

Briefly these genes describe a complex response: perception and signal transduction were characterized by the activation of HSP, transcription factors and RNA binding protein suggesting both transcriptional and post-transcriptional regulation. Moreover expression values have indicated a regulation of stomata closure as well as the activation of fatty acid degradation pathway.

Among these genes some drought and heat response gene markers have been identified and use to test the heat and drought response of the two cultivars at young developmental stages by qRT-PCR. The gene markers were selected according to the fold change threshold of the Affymetrix dataset. These sequences were considered ideal candidates for an eQTL mapping experiment aiming to identify the key loci controlling the molecular response to drought. The mapping experiment was based on the availability of a genetic map developed on the Ofanto x Cappelli Recombinant Inbreed Line (RIL) population, composed by 161 lines. Based on their expression profile, two genes were chosen to be tested on 80 RILs grown in water stress conditions by qRT-PCR. A highly significant (LOD=7) eQTL was identified on chromosome 6B which explained about 36% of observed variability for the expression of High Expression Level gene (HEL), one out of the two tested genes. HEL is a transcript, unknown annotated, shows a strong drought induction in Ofanto. The corresponding probe set matches with the contig444272 of Chinese spring draft genome assembly. The gene mapping is in progress in order to distinguish between a *cis*- or *trans*-action of the eQTL identified on the gene expression. Then, since this gene

is differentially regulated between the two cultivars contrasting for stress tolerance, additional experiments will be planned to map drought tolerance QTLs.

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Poster Communication Abstract – 2A.73

GENOMIC AND PROTEOMIC ANALYSES OF ZEINS IN INBRED LINES AND LOMBARD VARIETIES OF MAIZE

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Zea mays, zein, RFLP, 2D electrophpresis, barcoding

DNA barcoding, the use of a short standardised region of DNA for identifying either species and races, has been used successfully in animal field since 2003. However, optimisation of botanical barcoding has been more challenging. Although numerous strategies have been proposed, determination of the right stretch of plant DNA has been difficult.

In this study we exploited the possible use of *zein* genes for a rapid identification of maize lines and varieties. We choose *zeins* because they are a multigenic family (about 100 genes per haploid genome) that shows an extreme variability at the genic level and, consequently, a complex heterogeneity at protein level.

Zeins are storage ethanol-soluble proteins accumulated during seed development and they account for 50–70% of the total endosperm proteins of maize (*Zea mays*) and its wild ancestor *Teosinte*. Based on their solubility, their genetic properties and their apparent molecular masses, zeins have been classified into α- (22 and 19 kDa), β- (14 kDa), γ- (27 and 16 kDa) and δ-(10 kDa) zeins. The complexity level of peptide components in the zein fraction can be clearly shown by an analysis that combines the techniques of isoelectric focusing and SDS gel electrophoresis (2D electrophoresis).

We analysed zein fraction from several maize lines: the two well characterized lines at the genomic level (i.e. B73 and Mo17) and the four wild-type maize lines the most frequently used in previous genetic, biochemical and molecular analyses (i.e. BSSS53, W64A, W22, NYR and A69Y) together with some of their isogenic lines carrying mutant alleles affecting zein expression. These lines were used as reference to perform a systematic analysis of several Lombard varieties both at proteomic and genomic level of zein constituents. We obtain for each variety a distinctive 2D profile characterized by numerous isoforms within the molecular mass classes and charge for all zeins. These data constitute the *zein proteome profile* (zPP) that reveals a unique pattern for each variety. This indicates a high heterogeneity in the coding sequence most probably based on SNPs leading to amino acid substitutions of charged or polar residues. Sequence analyses of more than 150 zein genes confirmed the above hypothesis. At genomic level each maize line or Lombard variety manifests a unique RFLP pattern, *zein genome profile* (zGP).

Based on these results a specific set of primers has been developed for direct analysis by PCR multiplex methodology.

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Poster Communication Abstract – 2A.74

ANALYSIS OF POLYMORPHISMS BETWEEN *SBEIIA* HOMOEOLOGOUS GENES IN WILD AND CULTIVATED WHEATS

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Starch, starch branching enzyme, wheat

Starch branching enzymes play a crucial role in amylopectin synthesis determining the grade of branching of the glucan chains. SBEs cleave α-1, 4-glucosidic linkages and reattach released chains through α-1, 6-glucosidic linkages to the same or another α-1, 4-linked glucan chain.

While it is not completely clear the contribute of SBEI to starch structure, SBEII have been shown to be essential to maintain a normal ratio between amylose and amylopectin chains in cereals.

Bread wheat is an allohexaploid species that was formed through successive chromosome doubling of hybrids involving three ancestral diploid species *T. urartu*, *T. tauschii* and a species of the Aegilops section Sitopsis (Levy and Feldman 2004). *Aegilops speltoides* is commonly identified as the ancestral species more closely related to the wheat B genome but it remains unclear if the origin of B genome was monophyletic or originated from the introgression of several species. A more recent hypothesis suggests that several lines of *Aegilops speltoides* have contributed to the B genome formation of polyploidy wheats.

In this work the comparison of intronic regions of the three *SBEIIa* homoeologous genes showed the presence of several insertions/deletions. Several TE insertions were detected in B and D *SBEIIa* homoeoalleles.

Two insertions of 116 and 90 bp, localized respectively in intron XIV and XV, were identified in SBEIIa-B. Both of them resulted to be MITE (Miniature inverted repeat transposable) elements.

Two insertions of 130 bp localized in intron XI and of 150 bp in intron XVI of SBEIIa-D were classified as transposable elements by CENSOR analysis. The first sequence resulted similar to a transposon identified in *Triticum aestivum* (DNA-9-ta). The insertion in intron XVI resulted to be Thalos TA, a stowaway-like MITE belonging to the Tc1/mariner superfamily.

In order to establish new insights on the role of transposable elements in the evolution of polyploid wheats a collection of wild and cultivated polyploidy and diploid wheats was investigated to check the presence of the TEs identified in the bread wheat genomes.

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Poster Communication Abstract – 2A.75

PHYSICAL MAPPING OF GENOMIC AND EST-DERIVED SSR MARKERS ON THE HOMOEOLOGOUS GROUP 5 CHROMOSOMES OF WHEAT

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Durum wheat, cytogenetic map, 5A chromosome, microsatellites

The International Wheat Genome Sequence Consortium, aimed to physical mapping of bread wheat genome, assigned to Italian research groups the genetic and physical mapping of 5A chromosome. The aim of the present work was to find new molecular markers on chromosome 5A to saturate the existing genetic map, and to develop a cytogenetic deletion map of the homoeologous group 5. A set of 167 microsatellite markers identified in public databases as mapping on 5A and 5B chromosomes were physically mapped. In particular, 135 were genomic microsatellites (gSSR), 30 were derived from expressed sequence tags (EST-SSRs), and 2 were STS. Amplified bands were visualized by capillary electrophoresis performed using an ABI PRISM 3100 Avant Genetic Analyzer.

The SSR markers were physically mapped on 5A and 5B chromosome bins by using a set of aneuploid lines derived from the hexaploid wheat cultivar Chinese Spring (*Triticum aestivum*). Cytological mapping of microsatellite markers on 5A was conducted with nulli-tetrasomic lines CS-N5AT5B and CS-N5AT5D, the di-telosomic line CS-DT5AL, and 14 deletion lines of which 4 belonging to 5A short arm and 10 to the long arm. Besides, physical mapping of SSRs on 5B chromosome was carried out with the nulli-tetrasomic line CS-N5BT5D, the di-telosomic line CS-DT5BL, and 6 deletion bin lines of which 4 belonging to the chromosome short arm and 2 to the long arm. All the bin lines were tested for carrying the correct homozygous terminal deletions by PCR amplification with specific SSR markers belonging to the missing chromosome region.

Out of 167 analysed markers, 110 were physically mapped on specific 5A and 5B chromosome bins, while the other 57 were amplified both in 5A, 5B and 5D nulli-tetrasomic lines. A total of 46 SSR markers were assigned to chromosome 5A, 34 on 5B and 6 on 5D; 24 SSRs produced multiple loci which mapped to 5A and 5B (16), 5B and 5D (4), 5A and 5D (1) and 5A, 5B and 5D (3). For both the homoeologous the majority of markers were physically mapped in the bins of the long arm. Infact, the most represented bins were 5AL5-0.46-0.55 for 5AL and 5BL6-0.29-0.79 for 5BL, while the regions which were the most lacking of markers were represented by bin C-5AL5-0.46 and 5BS4-0.43-0.71, respectively for 5AL and 5BS. Physical mapping of SSR markers on 5A will provide a very powerful tool to anchor the 5A BAC sequence contigs to the chromosome.

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Poster Communication Abstract – 2A.76

STRUCTURAL ANALYSIS OF THE WHEAT GENES ENCODING NADH-DEPENDENT GLUTAMINE-2-OXOGLUTARATE AMIDOTRANSFERASES (NADH-GOGAT) AND COMPARISON WITH OTHER SPECIES

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NADH-GOGAT, wheat, 454-reads

Nitrogen uptake is an essential element in crop improvement and breeding for cereal cultivars that absorb and metabolize nitrogen most efficiently for grain or silage production. Maximizing efficient nitrogen utilization is becoming increasingly important for breeders. This aim requires a better understanding of nitrogen metabolism and its regulation, and identification of target genes to monitor N uptake by either direct gene transfer or marked-assisted breeding. One of the enzymes related to nitrogen metabolism is glutamine-2-oxoglutarate amidotransferase (also known as GOGAT). Together with glutamine synthetase (GS), GOGAT maintains the flow of N from NH_4^+ into glutamine and glutamate, which are then used for several other aminotransferase reactions during amino acid synthesis. We focused on *NADH-GOGAT* gene as one of the potential candidate genes for determining grain protein content (GPC). Using a rice *NADH-GOGATI* sequence as an initial query, we identified a GOGAT gene from the wheat B-genome within previously reported wheat genomic DNAs (Goto at al., 1998). We also extracted and assembled 454-reads of cv. Chinese Spring (http://www.cerealsdb.uk.net/search_reads.htm) and the 454-reads of a *Triticum tauschii* accession (http://avena.pw.usda.gov/RHmapping/blast/). From the single wheat gene sequence and 454 reads we were able to assemble the three orthologous genes from the three hexaploid genomes and from the D-genome ancestor *Triticum tauschii*. PCR primer pairs were design for the three distinct GOGAT hexaploid sequences and used to identify genome assignments using DNA from Chinese Spring nulli-tetrasomic lines for the group 3 chromosomes. A comparison of a set of plant *NADH-GOGAT* genes (wheat, *Brachypodium*, rice and sorghum) suggests regions of greater sequence and structure conservation likely related to critical enzymatic functions and metabolic control. We also obtained the *NADH-GOGAT* genomic sequence in the two Italian durum wheat cultivars Svevo and Ciccio, characterized by different grain protein content. Polymorphism between the two cultivars could be analyzed in an RIL population derived by crossing those two cvs and evaluating grain protein content in field trials. Such polymorphisms could then be used to verify the correlation with a QTL for GPC and assess the potential implication of *NADH-GOGAT* gene in controlling this very complex trait.

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Poster Communication Abstract – 2A.77

PROTEOMIC CHARACTERIZATION OF C-TYPE LMW-GS IN DURUM WHEAT

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C-type LMW-GS, durum wheat, 2-DE, PMF, PFF

C-type low molecular weight glutenin subunits (LMW-GSs) are components of the glutenin polymer (Molecular Weight 30-40 KDa) quantitatively present in a lower amount and studied to a limited extent, with respect to typical LMW-GS (also known as B-subunits). They are considered structurally gliadins but functionally glutenins, because they form inter-molecular disulphide bonds, result of mutations of their encoding genes that affect the number and/or the distribution of cysteine residues. N-terminal amino-acid sequencing confirmed that they are made up of α/β and γ-gliadin-like components encoded by genes present at the *Gli-1* and *Gli-2* loci. The presence of gliadin-like subunits in glutenin preparations, presumably have a negative effect on flour quality. Several studies have been addressed to investigate the structural features and the effects of the typical LMW-GSs on gluten strength, whereas less attention has been dedicated to the C-type group.

In order to characterize the specific polypeptides present in the C-type subunit group, two dimensional electrophoretic separation (2-DE) of an enriched fraction of C-type subunits from durum wheat cv Svevo has been carried out and selected protein spots from the gel were excised and subsequently analyzed by mass spectrometry. Peptide mass spectrometric data were used to perform protein identification in the Peptide Mass Fingerprint (PMF) mode and subsequently the peptide sequences information by the Peptide Fragment Fingerprint (PFF) approach to confirm the data obtained.

Results of proteins identified in the C-type glutenin subunit fraction will be presented.

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Poster Communication Abstract – 2A.78

EVOLUTIONARY CONSERVED STRESS-RESPONSIVE CCCH ZINC FINGER PROTEINS ARE INVOLVED IN GERMINATION PROCESSES IN *ARABIDOPSIS* AND DURUM WHEAT

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CCCH zinc finger domain, germination, salt stress, Arabidopsis, durum wheat

CCCH zinc finger domain consists of a sequence with three cysteines and one histidine residues with strictly defined spacing: C-X_{4-15}-C-X_{4-6}-C-X_3-H. First identified in proteins of Tristetraprolin family in mammals, involved in regulation of stability of cytokine mRNAs, this domain has been found in plant RNA-binding proteins involved in the control of important biological processes such as floral reproductive organ identity determination and calmodulin-mediated RNA processing.

A gene coding for a CCCH zinc finger protein, named 2H8, was isolated in durum wheat and characterised as responsive to cold and dehydration stresses (De Leonardis et al., 2007). Six additional cDNA sequences were identified in the wheat EST database following a similarity search. These genes were characterised by expression studies under cold and water stress conditions.

Overall, a family of more than sixty genes coding for CCCH proteins was recently described in Arabidopsis. A functional conservation between a sub-group of stress-related Arabidopsis CCCH genes and the seven highly homologous genes, identified in durum wheat as responsive to cold and drought stresses has been suggested.

To gain information of the role of this gene family in stress response, a functional analysis on the Arabidopsis genes via mutant analysis is underway. In particular, this study is focused on the characterization of a set of Arabidopsis lines generated by ihpRNA interference and amiRNA technologies resulting in the down-regulation of the CCCH zinc finger gene At4g29190 (the most homologous to the 2H8 gene of wheat).

A deep phenotypic evaluation of the germination process under abiotic stress conditions revealed that the mutant lines grow better than the wild type. These results suggest that the product of the At4g29190 gene operates as a negative regulator of germination in particular in presence of salt stress and low temperature. Experiments are in progress to test whether At4g29190 regulates the expression of the genes controlling the accumulation of GA and ABA genes whose activity is important in the germination process. A detailed expression analysis of the homologous wheat gene is also in progress to verify a possible involvement of this gene product in the germination of wheat seeds under stress conditions.

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Poster Communication Abstract – 2A.79

LINKAGE DISEQUILIBRIUM AND POPULATION STRUCTURE IN TETRAPLOID WHEAT

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Linkage disequilibrium, population structure, association mapping, durum wheat

Together with knowledge on population structure, a critical step for the planning of association and population genomics studies is the level of linkage disequilibrium (LD) that characterizes the specie and the population employed for the analysis. We have analyzed the population structure and the LD in a large collection of tetraploid wheats (*Triticum turgidum* subsp.) made of 128 accessions of durum wheat and 104 wild and domesticated accessions. All of the accessions were analyzed with 26 SSR and 821 DArT markers, most of which were genetically mapped. Our results partially reflect previous knowledge on population structure of tetraploid wheats, and they clearly show a sharp separation of durum wheat accessions from the rest of the naked and hulled tetraploid wheats. The population structure of durum wheat cultivars were in agreement the knowledge on the breeding history. Indeed, a strong correlation was found between the genetic structure of modern varieties and year of release. Landraces and wild accessions had a higher allelic diversity than modern durum wheat varieties for both genomes and all chromosomes in terms of total number of alleles and allelic richness. The wild accessions were characterized by very low levels of LD, while a higher LD value was observed for the subgroup containing the durum wheat genotypes (8.2, $P<0.001$). Wild and domesticated accessions represent a useful rich source of useful alleles for plant breeding and a powerful tool to detect and identify useful genes using association mapping and population genomics studies.

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Poster Communication Abstract – 2A.80

DEVELOPMENT OF A HIGH-DENSITY CONSENSUS MAP IN DURUM WHEAT

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Durum wheat, consensus map, segregation distortion, chromosomal rearrangements

A well-saturated genetic map is an important tool for plant breeding and many technologies are available to increase the abundance of molecular markers suitable for genetic analysis, such as DArT markers.

A consensus map of durum wheat (*Triticum turgidum* L. var. *durum*) was constructed based on segregation data from six mapping populations, including Creso x Pedroso, Ofanto x Cappelli, Cirillo x Neodur, Svevo x Ciccio, Messapia x MG4343 and Latino x Primadur. All listed genotypes are durum wheat varieties, except MG4343, which is an accession of *Triticum turgidum* var. *dicoccoides*. The composite map contained a total of 1916 markers, comprising SSR, EST-SSR, STS, TRAP, RFLP, morphological and biochemical markers. The total map length spanned 3021 cM spread over 25 linkage groups and showed a mean distance between neighbouring loci of 1.6 cM. Among all markers, 640 were common at least in two populations while 1276 were mapped in a single population. The comparison of marker order in the consensus and the individual maps, revealed a good co-linearity, except for few putative inversions in the frame of few cM.

A small fraction (8%) of the markers deviated significantly from the expected Mendelian ratio; clusters of loci showing distorted segregation ($P<0.01$) were found on chromosomes 5A, 6A, 1B, 2B, 4B, 5B, 6B, and 7B.

The analysis of map location of putative homoeologous loci suggest the occurence of several rearrangements in chromosomes 4A, 5A, 6A, and 7B. The putative translocation on chromosome 7B was previously described.

This consensus map represents a very useful tool, providing a more complete coverage of the durum wheat genome, to facilitate genomic researches such as evolutionary studies, QTL fine mapping for map-based cloning, evaluation of the degree of linkage disequilibrium and association analysis of important agronomic traits.

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Poster Communication Abstract – 2A.81

NEW SNPs MUTATIONS OF DREB GENES IN DURUM WHEAT IDENTIFIED BY HRM TECHNOLOGY

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Salt stress tolerance, DREB genes, High Resolution Melting, durum wheat

DREB (Dehydratation-responsive-element binding factor) is one of the largest families of transcriptional regulators and form an integral part of signalling webs which modulate many plant processes, such as abiotic stresses tolerance. DREB genes are induced by low temperature, salinity and drought, they contain conserved EREBP/AP2 domains and the DRE-motif functions as a DRE-binding transcription factor sequences. In the present paper an innovative method has been applied to identify novel alleles in DREB genes family (DREB-1, DREB-2, DREB-3, DREB-4, DREB-5 and DREB-6 gene) in four salt and drought resistance and susceptible *Triticum durum* lines (Cham-I, Jennah Khetifa, Belikh 2 and Trinakria). This technique involves scanning for sequencing variations in cDNA-derived PCR amplicons using High Resolution Melting (HRM) followed by direct Sanger sequencing of only those amplicons which were predicted to carry nucleotide changes. High Resolution Melting represents a novel advance for the detection of SNPs measuring temperature-induced strand separation of short PCR amplicons. The use of this approach is still limited in the field of plant biology. Here, HRM analysis has been applied to the discovery and genotyping of durum wheat SNPs. Specific primers have been designed, starting at multi-alignment of DREB genes conserved portions. The PCR amplicons, containing SNPs, produce distinctive HRM profiles, and by sequencing the PCR products identified, SNPs have been characterized and validated. The results showed that all the identified SNPs are located on salt tolerant variety J. Ketifa treated with the maximum salt concentration (1.5 M) confirming its value in breeding activities. Moreover, all SNPs mutations correspond to amino acid changes in the conserved portion of DREB genes probably influencing protein activity and function.

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Poster Communication Abstract – 2A.82

CHARACTERIZATION OF WHEAT LOW-MOLECULAR-WEIGHT GLUTENIN SUBUNITS AND THEIR MATURATION PROCESS

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Wheat, LMW-GS, maturation process, asparaginyl peptidase, proteomics

The glutenin polymers of wheat endosperm are made up of two main types, the high- (HMW-GS) and low- (LMW-GS) molecular weight subunits; subtypes of LMW-GS include LMW-m, LMW-s, and LMW-i, named according to the first amino acid of the mature sequence.

The main difference between LMW-m and LMW-s is the absence in the mature LMW-s type of the expected first 3 N-terminal amino acids (MET-) characteristic of the LMW-m type. According to DNA sequences, however, a nucleotide sequence corresponding to 3 amino acids is present in the sequences encoding both the precursors, although rather than being MET, the precursor sequence of LMW-s is MEN.

According to algorithms that predict the signal cleavage site, the signal peptidase should generate a QMET N-terminal sequence and removal of the N-terminal Q must occur in order to generate the m-type LMW-GS, unless the prediction is incorrect and the N-terminal Q is actually where the signal cleavage occurs. The presence of N instead of T in LMW-s was the basis for our hypothesis that a differential processing of the N-terminal end of the LMW-s sequence occurs such that cleavage at the N residue by an asparaginyl peptidase generates the observed N-terminus of the LMW-s type, similar to the processing that apparently occurs in ω-gliadins.

In order to investigate this possibility, we produced transgenic wheat lines, transformed with mutated versions of the LMW-m and LMW-s genes, such that N was substituted for T in the LMW-m gene and T for N in the LMW-s gene, for comparison with their wild type counterparts. Western Blot analyses on 2D gels and proteomic comparisons between the transgenic and untransformed lines, have allowed to identify the transgenic polypeptides. MS-MS analyses have indicated that the processing occurs according to our predictions.

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Poster Communication Abstract – 2A.83

IDENTIFICATION OF CHROMOSOME 5A ENCODED POLYPEPTIDES IN WHEAT KERNELS

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Wheat, chromosome 5A, proteomics, kernel proteome

The wheat endosperm is composed for 80% of storage proteins which are mainly responsible of dough technological properties. The remaining 20%, the soluble fraction, includes most of the metabolic proteins. If gluten proteins are coded mainly by genes present on the chromosomes 1 and 6, the chromosome location of soluble proteins, including polypeptides with structural and metabolic functions, still needs to be identified and mapped. Genes present on the chromosome 5 identified so far have a role in the quantity of protein, in the frost resistance, and in the kernel hardness.

A procedure to extract specifically the different wheat kernel protein classess has been applied to different genetic lines, such as intervarietal and interspecific chromosome substitution lines, in order to perform two-dimensional proteomic maps allowing to attribute the chromosome localization of specific polypeptides to the 5A chromosome.

We first analysed the interspecific substitution line in which chromosome 5A of the durum wheat Langdon has been replaced by chromosome 5A of *T. dicoccoides*.

For the metabolic fraction, the analysis made on mature seeds of durum wheat, revealed 22 spots corresponding to polypeptides encoded by genes on chromosome 5A of *Trititcum dicoccoides* and 10 spots corresponding to polypeptides encoded by genes on chromosome 5A of *T. turgidum* cv Langdon. Among these, the xylanase inhibitor XIP III was found, that has an important role on quality properties.

Concerning the Chloroform-Methanol fraction the analysis revealed 6 spots corresponding to polypeptides encoded by genes on chromosome 5A of Trititcum dicoccoides and 3 spots corresponding to polypeptides encoded by genes on chromosome 5A of T. turgidum cv Langdon.

As regards the use of intervarietal 5A chromosome substitution lines, five lines in which the 5A chromosome of the bread wheat cultivar Chinese Spring has been replaced by each of 5A chromosomes of cultivars Hope, Thatcher, Timstein and Cheyenne, are under investigation.

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Poster Communication Abstract – 2A.84

TOMATO TRANSCRIPTOME IS REPROGRAMMED FOLLOWING PROSYSTEMIN OVER-EXPRESSION

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Systemin, plant-insect interactions, microarray

In tomato plants, the response to insect attacks and mechanical wounding is mediated by systemin (Sys), an 18-aminoacid signaling peptide derived from the precursor protein called prosystemin (ProSys). Sys is considered the primary wound signal that, once induced, triggers the production of jasmonic acid (JA) via the octadecanoid pathway. JA activates, locally and sistemically, the expression of defensive genes, such as proteinase inhibitors and polyphenol oxidase that prevent uptake of essential amino acids in insect gut, thus causing negative effect on pest development. To study the impact of prosystemin expression on tomato transcriptome, transgenic plants over-expressing ProSys were produced (RSYS) and analysed by microarray. RSYS plant population was screened to assess transgene presence, its expression and protein production.

Two transgenic genotypes, differing in ProSys expression level, were chosen and each one was used in three biological replicates. A two colour-labeling strategy was used for a competitive hybridization of samples and controls on tomato Agilent 4x44k array. Image data were processed using Agilent Feature extraction software and data analyses were performed using GeneSpring GX 10. Blast2GO software (CIPF, Valencia) was used for the functional annotation of differentially expressed sequences. Most of them are classified in "stress responses" and "signaling": ProSys was found to affect the expression of genes related to defence against herbivores and pathogens, including genes involved in the reinforcement of physical barriers, and environmental stresses. Moreover, the expression of genes related to the emission of volatile compounds, associated with indirect defence mechanisms, such as phenylpropanoid and terpenoid, was also affected. Interestingly genes involved in jasmonic acid, salicylic acid, ethylene and auxin-regulated pathways were differentially expressed, suggesting that Sys could influence the defence networks controlled by different plant hormones. Overall our results indicate that tomato Sys plays a key role in plant defence against different stressors.

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Poster Communication Abstract – 2A.85

GENOMIC STABILITY OF ANDROGENETIC HAPLOIDS DERIVED FROM *SOLANUM TUBEROSUM (+) S. BULBOCASTANUM* SOMATIC HYBRIDS

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Potato, molecular markers, nuclear DNA, cytoplasmic DNA

Somatic hybridization via protoplast fusion provides a powerful tool to overcome crossing barriers in potato (4x=48), allowing the integration of parental nuclear and cytoplasmic genomes. Previously, we used protoplast fusion to produce hybrids between incongruent 2x *S. bulbocastanum* and haploids of the cultivated potato *S. tuberosum*. Due to somatic hybrid sterility, we engineered haploidization as strategy to overcome such a drawback. The aim of this study was to establish the extent of genetic variation of somato haploids obtained through anther culture. The assessment was carried out at mitochondrial (*mt*) and chloroplast (*cp*) level by 13 (4 *mt* and 9 *cp*) ‘‘universal primers’’ homologous to conserved sequences, and at nuclear level by 8 Inter Simple Sequence Repeats (ISSRs) markers. As for the cytoplasm, the analysis revealed a very low rate of polymorphism in our haploids both for *cp*- and *mt*-DNA. This result is likely due to the very strong stability of such genomes. By contrast, a high degree of polymorphism was detected at nuclear DNA level, ranging from 17% to 38%. Pair-wise comparisons between the banding patterns of haploids and somatic hybrids they derived from allowed detecting two types of changes: disappearance of parental ISSR fragments (termed “loss”) and appearance of novel ISSR fragments (termed “gain”). The most frequent event occurring in the haploids was the loss of fragments (16% on average). Cluster analysis revealed that haploids were genetically distant from the parental somatic hybrids as well as among them. Implications of our findings from a breeding standpoint will be discussed.

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Poster Communication Abstract – 2A.86

STRUCTURAL GENOMICS OF WILD POTATO SPECIES BASED ON DArT ALIGNMENTS

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Wild potato species, Solanum, molecular markers, bioinformatics

To improve the access to resistance and agronomically significant genes of *Solanum bulbocastanum* and *S. commersonii*, two wild potato species, we had previously developed their first genetic linkage map based on DArT markers. In this study, bioinformatics and ongoing sequencing efforts for the potato (*Solanum phureja*) and tomato (*Solanum lycopersicum*) genomes were useful to select DArT markers associated with interesting genes. About 1500 DArT markers, representing the *S. bulbocastanum* and *S. commersonii* maps, were sequenced by BigDye™ Terminator ABI PRISM® 310 Genetic Analyzer. Then, they were aligned using the GenomeThreader software along the chromosome sequences of both potato (http://www.potatogenome.net) and tomato (http://solgenomics.net) genomes. Although a filter of 70% of coverage and score was considered, most of alignments showed a quality higher than 90%. Ninety-two % of DArT markers aligned on the potato genome, while 79% of them aligned to tomato. In particular, only the 5% of markers aligned to neither genome, thus revealing makers potentially specific to genomic regions of the wild potato species. As expected, DArT markers showed better correspondences with the potato. Interestingly, in both genomes DArT markers were preferentially distributed on chromosomes I, II, III, IV and VI. Among DArT markers belonging to linkage groups and aligned to both the potato and the tomato genomes, collinearity as well as correspondence with annotated genes were found. Moreover, the chromosome association of the linkage groups analyzed was considered. Results are discussed from evolutionary and breeding standpoints.