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**Poster Communication Abstract – 9.01**

# TESTING HIGH-RESOLUTION MELTING FOR SNP DISCOVERY AND GENOTYPING IN DIPLOID AND POLYPLOID CEREALS

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*HRM, SNPs, TILLING, durum wheat, barley*

High Resolution Melting (HRM) is emerging as a novel technique for single-nucleotide polymorphism (SNPs) discovery and genotyping. With HRM, allelic differences are detected based on differences in melting temperature using a DNA intercalating dye. This makes HRM a fast, simple and relatively cheap close-tube technique which does not require expensive chemistry for probe labelling and any processing after PCR.

In order to explore the potential of HRM technique in both diploid and polyploid species, a composite study was performed in barley (*Hordeum vulgare* L.) and durum wheat (*Triticum turgidum* L.*)*. We first addressed the sensitivity of HRM for SNP discovery by screening several amplicons containing known SNPs of all types (AT, AG, etc). In barley, the sensitivity reached at least one mutated copy out of 20 W.T., equivalent to 1 heterozygous plant in 10-plant bulks. Such sensitivity makes HRM fully suitable for TILLING (or EcoTILLING)-like mutation discovery approaches. In durum wheat, an HRM-based protocol was successfully tested for SNP discovery at target loci, in conditions where both homoeologous target sequences were amplified. We also tested the HRM suitability for genotyping of known SNPs and indel markers in durum wheat. When experimental biparental populations were utilized, HRM provided fully accurate genotyping results, including heterozygous SNPs in the presence of a second homoeolog amplicon. However, HRM profiles were difficult to interpret when applied to a population segregating for multiple alleles.

Based on our results, HRM confirmed to be a potentially interesting technique for molecular marker genotyping for mapping, marker-assisted selection and/or TILLING.

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**Poster Communication Abstract – 9.02**

# IMPROVING THE TECHNOLOGY FOR FLOW SORTING WHEAT 5A CHROMOSOME ARMS SPECIFIC DNA

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*Triticum aestivum, flow cytometry, FISH*

Common wheat (*Triticum aestivum* L. 2n=6x=42 AABBDD) is one of the most important commodity. It is an hexaploid species with a huge genome of 17 Gbp composed for more then 80% of repetitive DNA sequences. Three homeologus genomes and large interdispersed repetitive sequences make genomics of wheat a challenging task. Flow sorting of specific chromosomes and chromosome arms in suspension is a useful and handly approach to dissect complex genomes, on the basis of a smaller amounts accounting for only a few percent of the whole nuclear DNA content. Flow-sorted chromosomes are an invaluable source of DNA that can be used for physical gene mapping, isolation of molecular markers, and construction of chromosome-specific DNA libraries. Here we describe the procedure to obtain 5AS chromosome arm specific DNA by flow cytometry analysis, flow sorting, proteinase K digestion and isothermic DNA amplification. All the primary steps have been optimized in terms of analysis accuracy, time and yield such as: (1) accumulation of cells in metaphase, (2) preparation of chromosome suspensions, (3) flow analysis and sorting, (4) purity control of sorted chromosomes and (5) processing of their DNA. In particular, we have: improved the precision of DAPI chromosome staining; applied a simple and fast FISH method for purity estimation of sorted fractions; adopted a faster processing method to purify and obtain chromosomes specific DNA of high molecular weight. These new achivements will contribute to make easier and more effective the chromosome approach for wheat and other crop.

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**Poster Communication Abstract – 9.03**

# *LOLIUM PERENNE TERMINAL FLOWER 1* GENE EXPRESSION IN ALFALFA AND TOBACCO DOES NOT AFFECT FLORAL TRANSITION

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*TERMINAL FLOWER1 gene, Medicago sativa, Nicotiana tabacum, floral transition*

Understanding and controlling the flowering process is extremely interesting for its important agronomic implications. In forage crops, like alfalfa, the delay or the suppression of flowering could have a positive effects on forage quality. In addition, the manipulation of flowering transition could allow to prevent gene flow and dispersal of transgenes from genetically engineered alfalfa. Genetic and molecular analysis have shown that several genes are involved in the control of the switch from vegetative to reproductive growth. Recently, the TERMINAL FLOWER 1 gene of *Lolium perenne* (*LpTFL1*) has been isolated and overexpressed in *Festuca rubra* and *Arabidopsis thaliana.* In some transformation events, the complete suppression of flowering was reported. We have evaluated the *LpTFL1* GENE as a possible candidate to control the flower transition process in two genetically distant plants: alfalfa and tobacco. The two species have been transformed via *Agrobacterium tumefaciens* using the original binary vector pCAMBIA3300-*LpTFL1* (kindly provided by C.S. Jensen), in which the *LpTFL1*gene was placed under the control of the *Zea mais Ubiquitin* promoter (ZmUBI) and the *Nos* terminator (Nos). Furthermore, to ensure a high level of expression of the gene, a second expression cassette was developed replacing the promoter *ZmUBI* with the cauliflower mosaic virus (CaMV) *35S* dual-enhancer promoter. The transformation with the binary vector pCAMBIA3300-*LpTFL1* produced 13 and 12 transgenic plants in alfalfa and tobacco respectively. RT PCR analysis performed on the positive transgenic plant confirmed the expression of *LpTFL1*gen in both species. No effect on floral transition or flowering was observed. Alfalfa and tobacco transgenic plants were phenotypically normal throughout the growth cycle, and set seed normally.

Transformation with the *35S*-driven gene is underway to confirm whether *LpTFL1* can influence the floral transition of alfalfa and tobacco.

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**Poster Communication Abstract – 9.04**

# HORDOINDOLINE COMPOSITION AND KERNEL HARDNESS IN BARLEY (*HORDEUM VULGARE*)

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*Barley, kernel texture, hordoindolines*

Grain hardness has a strong impact on the end-use quality of barley because good-malting cultivars are softer in texture than poor-malting genotypes, whereas harder textured varieties are desirable for use as feed. Barley kernel texture and related traits such as milling energy, particle size, starch damage, malt extract yield and digestibility in ruminants, are modulated by Hordoindoline A (Hin-A), Hordoindoline B1 (Hin-B1) and Hordoindoline B2 (Hin-B2) encoded by *Hina*, *Hinb-1* and *Hinb-2* genes on the short arm of chromosome 5H, respectively. A-PAGE and A-PAGE x SDS-PAGE fractionations of starch granule proteins from 27 barley cultivars with contrasting grain texture characteristics revealed three pairs of prominent polypeptides, approximately 15 KDa in size, which were assumed to correspond to Hin-A, Hin-B1 and Hin-B2 on the basis of the electrophoretic patterns of some peculiar genotypes possessing modified or null hordoindoline alleles. Cvs Sundance and Hart, which were claimed to be unique in lacking Hin-A, were found to possess different *Hina* alleles and accumulate Hin-A on their starch granules. Two novel alleles for *Hinb-1* and *Hinb-2* were detected from barley cvs Steptoe and Sundance, respectively. Ten two-rowed and ten six-rowed cultivars grown in replicated plots were compared for kernel weight and hardness, as determined by the Single Kernel Characterization System (SKCS) applied to their whole or pearled grains. No significant difference in SKCS index was found between whole and pearled kernels. On the contrary, pearled kernels from two-rowed cultivars were significantly softer (mean SKCS = 66.2± 5.6) than those from six-rowed genotipes (77.0±8.0). A significant negative correlation of -0.75 was observed between SKCS index and kernel weight, the difference in this latter trait explaining approximately 25% and 50% of the phenotypic variation for kernel hardness in two-rowed and six-rowed cultivars, respectively.

The relationship between the presence of certain hordoindoline alleles and grain texture is discussed.

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**Poster Communication Abstract – 9.05**

# CLONING AND CHARACTERIZATION OF THREE HOMOEOLOGOUS WHEAT PDI-LIKE GENES LOCATED ON GROUP 5 CHROMOSOMES

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*Gene structure, gene promoter, Triticum, protein folding, wheat quality*

PDI and PDI-like proteins are responsible for multiple metabolic functions, including secretory protein folding, chaperone activity and redox signalling. In plants the proteins of the PDI family cluster into eight phylogenetic classes, five of them include proteins with two thioredoxin (TRX)-like active domains, whereas the other three classes own a single TRX-like domain. The first class includes the typical PDI, which in cereals may be involved in the folding of secretory proteins during the formation of endosperm protein bodies. The three homoeologous genes coding for the typical PDI and their promoter sequences had previously been isolated and characterized. Their exon/intron structure is highly conserved and includes 10 exons. Recently we reported the characterization of the whole set of nine non-homoeologous PDI-like gene sequences of wheat; since phylogenetic analysis assigned them to the eight plant subfamilies, at least one wheat gene has been cloned for each group. In this study we report the characterisation of the genomic and cDNA sequences of three homoeologous PDI-like genes (TaPDIL5-1A, TaPDIL5-1B and TaPDIL5-1D) of the V phylogenetic group and located in chromosome arms 5AL, 5BL and 5DL of hexaploid wheat. The coding region and the exon/intron structure, consisting of nine exons, of their genomic sequences were highly conserved. The most relevant differences were detected in the length of the second (1393 bp in TaPDIL5-1B, 1076bp in TaPDIL5-1A and 1026 bp in TaPDIL5-1D) and fifth (967 bp in TaPDIL5-1D, 723 bp in both TaPDIL5-1A and TaPDIL5-1B) introns. Their ORFs consisted of 1323 bp, corresponding to polypeptides of 440 aa, with an estimated Mw of 47.2 KDa and pI of 5.3. The three encoded proteins possessed two tandem TRX active domains (***a°-a),*** each containing the typical tetra-peptide site -CGHC-, an inactive TRX ***b*** domain at its C-terminus, the signal peptide and a modified NDEL signal for retention in the ER. The comparison of the wheat sequences with the PDI-like genes of the V phylogenetic group from *Ararabidopsis*, rice and the moss *Physcomitrella patens* revealed a high level of conservation of their structural features, in terms of intron pattern and exon number, size and position of the active sites. The promoter sequence of the PDI-like gene located in 5A chromosome of bread wheat cv Chinese Spring was cloned using the inverse PCR (IPCR) tecnique. The sequence analysis showed that the fragment cloned by IPCR included about 1400 bp located upstream of the coding sequence. The promoter sequences of the PDI-like genes located in 5B and 5D chromosomes of C. Spring were cloned through PCR amplification using two primer pairs. One of the primers, the same for both pairs, was designed on the basis of a sequence in the distal region of the previously cloned promoter of the 5A chromosome, whereas the second specific primer of each pair was chosen within regions of the

second intron. The search of cis-acting regulatory elements within the promoters of the three genes was performed using the databases of plant promoters PlantCARE and PLACE and the differences between the three sequences will be discussed. Finally, transgenic durum wheat lines (cv Svevo) over-expressing the gene located in 5A chromosome, which was put under the control of the ubiquitin promoter of maize, were produced for the functional characterization of this gene. PCR analyses of 40 plants regenerated from 800 bombarded embryos showed that 5 of them were transgenic for the PDI over-expression construct.

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**Poster Communication Abstract – 9.06**

# ISOLATION AND CHARACTERIZATION OF APR (ADENOSINE 5' - PHOSPHOSULFATE REDUCTASE) AND APR-LIKE GENES IN WHEAT

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*Sulfate assimilation, sulfur metabolism, gene structure, Triticum, wheat quality*

The thioredoxin (TRX) superfamily includes redox proteins sharing a common structural motif named thioredoxin domain or "thioredoxin fold" with a consensus catalytic site sequence CxxC/S. The secondary structure of TRX domain consists of 5 β-strands surrounded by α-helices forming a central pleated β-sheet. The plant TRX superfamily is extremely more complex than in other eukaryotes. Phylogenetic analysis of 104 Arabidopsis protein sequences containing TRX domains formed five major clades, consisting of subfamilies with putatively distinct functions: thioredoxins, glutaredoxins (GSXs), protein disulfide isomerases (PDIs), peroxiredoxins and ferredoxins. The major clade containing PDI and PDI-like proteins included also two groups having different enzymatic activities. One of these consisted of two proteins of the quiescin-sulfhydryl oxidase (QSOX) family, which associate an oxiding TRX domain with an FAD containing ERV domain. The other group included seven proteins, three of them containing a TRX domain and a domain responsible for adenosine 5'- phosphosulfate (APS) reduction. These three proteins belong to the adenosine 5'- phosphosulfate reductase (APR) family, including key enzymes for plant sulfate assimilation through reduction of APS to sulphite. The four remaining APR-like proteins showed significant homologies and structural similarities with the TRX domains of APR but lacked the APS reductase domain.

The aim of this study was the cloning and characterization of APR and APR-like genes in wheat. A BLAST search of the DFCI Wheat Gene Index (TaGI, version 12) database using the seven available sequences of *APR* and *APR*-like genes of Arabidopsis and the six sequences of rice fetched four distinct contigs (TC, tentative consensus sequences), which were used for cloning the full-length cDNAs of four non-homoeologous APR and APR-like wheat genes: *TaAPRL1*, *TaAPRL3*, *TaAPRL5* and *TaAPRL6*. Southern and PCR analyses of DNA from Chinese Spring and its nulli-tetrasomic lines showed that the three homoeologous sequences of the four genes were located in the following chromosome homoeologous groups: 1) *TaAPRL1,* group 2; 2) *TaAPRL3*, group 3; 3) *TaAPRL5,* group 5*; 4) TaAPRL6,* group 7. The search in different protein databases for conserved motives in the deduced amino acid sequences of the four non-homoeologous genes revealed significant structural differences between the protein encoded by *TaAPRL1* and the three remaining APR-like proteins. TaAPRL1 (460 aa) was the largest among the APR-like proteins identified in wheat (TaAPRL3: 317 aa; TaAPRL5: 300 aa; TaAPRL6: 300 aa) and, in addition to a C-terminal TRX domain, it possessed the domain responsible for APS reduction and a chloroplast/plastid transit peptide sequence at the N-terminus. The remaining three APR-like proteins showed a high level of conservation of their structural features, in terms of both size and domain composition. They had a central TRX domain, a C-terminal trans-membrane segment and an N-terminal signal peptide. The structural analysis of the deduced amino acid sequences of the

four isolated genes suggests that only TaAPRL1 (the true wheat APR protein) is involved in the sulfate assimilation pathway, whereas the other three proteins (APR-like) may be implicated in redox reactions within the secretory pathway. Genomic and cDNA sequences of the three homoeologous *TaAPRL1* genes were cloned, sequenced and the most relevant differences are discussed.

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**Poster Communication Abstract – 9.07**

# EVIDENCE OF HERBICIDE TOLERANCE GENE FLOW FROM CULTIVATED CLEARFIELD RICE (*ORYZA SATIVA* L.) TO RED RICE (*ORYZA SATIVA* F. *SPONTANEA*)

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*Herbicide tolerance, clearfield rice, AHAS-inhibiting herbicides, gene flow, red rice*

The weedy relative of cultivated rice, red rice, can invade and severely infest rice fields, both lowering yields and reducing the selling price of the harvested grain. Infestations caused by this weed have been reported by rice farmers throughout the world. Because of its close genetic relationship to commercial rice, red rice has proved difficult to control. No herbicide yet developed can adequately control red rice without also injuring or killing conventional rice. Clearfield rice, which is resistant to the chemical group of AHAS-inhibiting herbicides called imidazolinones, has been developed by treating seeds of cultivated rice with the chemical mutagen ethyl methanesulfonate (EMS) and selecting for the resulting herbicide tolerant plants. Clearfield rice provide an high efficient opportunity to control red rice infestations and in order to reduce the risk of herbicide resistance spreading from cultivated rice to red rice Stewardship Guidelines are regularly released. Five years ago (2006) Italy started the cultivation of the Clearfield cultivar Libero. During the year 2010 a surveillance of the possible escape of herbicide resistance has been carried out. Red rice plants have been sampled at the edges of fields actually cultivated or previously cultivated with Libero. The collected plants have been analyzed for the presence of herbicide resistance by herbicide treatment and by molecular analysis of the region of the AHAS gene in search for the nucleotide variation determining the tolerant phenotype. The results showed clearly that in field are already present herbicide tolerant red rice plants, moreover the finding of plants homozygote for the mutation suggested that the cross event is happened minimum two years ago and that these plants are in the F2 generation or further.

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**Poster Communication Abstract – 9.08**

# MOLECULAR TECHNIQUES FOR THE FINGERPRINTING OF DURUM WHEAT VARIETIES

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*Durum wheat, fingerprinting, fAFLP, SSR-microsatellite, RAPD*

The use of biotechnologies has provided additional tools to monitor and trace the cereal chain, through DNA analysis. The aim of the study was to compare three molecular techniques: fAFLP (*Amplified Fragment Length Polymorphism* in fluorescence), SSR (*Simple Sequence Repeat*) and RAPD (*Random Amplified Polymorphic DNA*).

The use of fAFLP technique shows some advantages in the identification of diagnostic or specific markers. Although these markers are generally dominants, the AFLP analysis does not require previous knowledge of the DNA sequence, generates reproducible fingerprinting profiles and allows the amplification of a high number of DNA fragments per reaction, enabling the detection of specific amplified fragments. The AFLP fragments are usually scored as presence or absence of bands among a set of genotypes. The technique was developed for application to durum wheat and risulted effective to identify low levels of genetic variability and to discriminate between genetically similar genotypes.

The SSR technique is "single-locus type" and co-dominant markers distinguish homozygous from heterozygous loci, respectively represented by only one band or two bands. In this study, 13 microsatellites were selected and the discrimination ability was evaluated by checking their efficiency in distinguishing among 20 accessions of durum wheat. The RAPD analysis involves the use of a single decamer primer with random sequence and the number of polymorphisms obtained for each primer varied from 6 to 12. This technique is very effective for homogeneity test and can be usefully adopted to check for contamination, especially in the seed industry.

The three methods considered can be useful to protect the authenticity and traceability of the cultivars in the cereal chain.

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**Poster Communication Abstract – 9.09**

# ISOLATION OF PROTOPLASTS FROM MESOPHYLL CELLS OF *DENDROBIUM*

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*Dendrobium, mesophyll cells, protoplasts*

This study is part of the Project NOVAORCHID designed to explore the possibility to create intergeneric somatic hybrids of orchids by protoplasts culture technology. Our work is mainly focused on the genus *Dendrobium*, one of the largest genera in the orchid family Orchidaceae. It occupies a foremost position in ornamental orchid cut flower industry because of its high number of flower per inflorescence and recurrent flowering. The demand of the international market for *Dendrobium* cut flowers is, therefore, continuously increasing.

Somatic hybridization through protoplast fusion offers the opportunity to broaden the genetic variability among *Dendrobium* and generate cultivars showing new, fascinating and persisting flowers.

Plants of *Dendrobium spp* were obtained after *in vitro* cultivation of protocorm-like bodies (PLBs) and from vegetative micropropagation of selected genotype. PLB were subcultured on a modified MS medium. Young leaves of plantlets regenerated from PLBs were used as the explants for protoplast isolation. In order to obtain the optimal conditions for protoplast isolation several procedures were tested. Three different kinds of enzyme solutions together with three different incubation times were examined. After digestion a new and effective method of protoplast suspension has been set up. High number of purified protoplasts were collected and their vitality was tested.

In conclusion, an efficient procedure for *Dendrobium* protoplast isolation and culture conditions is described by adjustment of different steps which result in the enhancement of protoplast yield.

By utilizing the method developed in the present study we are now carrying a large programme of somatic hybridization involving *Dendrobium* and *Phalaenopsis* orchid species.

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**Poster Communication Abstract – 9.10**

# BIOTECHNOLOGICAL APPROACHES TO THE GENETIC IMPROVEMENT OF *CHRYSANTHEMUM CINERARIAEFOLIUM* L.

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*Protoplast, Chrysanthemum cinerariaefolium L., Asteraceae*

The interest in sustainable agriculture has increased the demand of plant-derived compounds which can be less toxic both to mammals and to the environment than the synthetic agrochemicals.

*Chrysanthemum cinerariaefolium* L. (Asteraceae), commonly termed pyrethrum, is an economically important crop from highlands of tropical and subtropical regions of the world. It is grown for the extraction of pyrethrins, natural insect repellents of plant origin. Pyrethrins are a mixture of six compounds produced by esterification of two acids (chrysanthemic and pyrethric acid) with three mono-terpene-alcohols (pyrethrolone-5, jasmolone-3 and cinerolone-4). The principale source of pyrethrins are the dried flowers of *Chrysanthemum cinerariaefolium*. Thanks to the low toxicity to mammals and other warm blooded animals, pyrethrum is the only plant species whose metabolites are currently commercially exploited in insecticides, and its worldwide demand exceeds the supply.

Asteraceae species are considered as recalcitrant to successful growth in *in vitro* condition. Recalcitrance in root or shoot formation or in regeneration are associated to endogenous bacterial contamination, hyperhydricity, and tissue browning, hence studies on *in vitro* culture of *Chrysanthemum cinerariaefolium* have been rather limited. The aim of this study was to establish highly reproducible *in vitro* regeneration systems for an efficient multiplication of *Chrysanthemum cinerariaefolium,* since the pointing out of an efficient regeneration system could play an important role for the industrial exploitation of this plant.

Petiole explants and leaf segments were used for micropropagation, callus induction and protoplast isolation. Sterile seeds were used as plant material source. The ability of petiole cuttings to produce direct shoot buds varied depending upon the different media composition tested for the experiments. Growth rate of shoots as well as root induction from shoots have been periodically analyzed. The *in vitro* raised plantlets were acclimatized and transferred to greenhouse with 60% success.

For protoplast isolation and culture, young leaves of *in vitro* grown plants were used as initial plant material. Different enzymatic combinations were tested to achieve the highest protoplast release. We were able to recover a reasonable number of viable protoplast for further manipulation at the ploidy level with the aim to enhance the biological activity of *Chrysanthemum cinerariaefolium* and for the regeneration of novel insecticidal plant germplasm.

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**Poster Communication Abstract – 9.11**

# POLYPLOIDY INDUCTION AND PROTOPLAST ISOLATION FROM PROTOCORM LIKE BODIES IN ORCHIDS

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*Dendrobium, flow cytometry and sorting, polyploidization, amiprophos-methyl, colchicine*

*Orchidaceae* is one of the most highly developed monocotyledonous families, their pot plant and cut flower production have high economical value in international flower markets. So the genetic improvement in orchids is very important to obtain new varieties and hybrid combinations commercially attractive. One way to introduce new variability is to double the ploidy level of the wild and commercial varieties. Polyploidy is a very useful tool in ornamental plant breeding. It may allow to overcome barriers to hybridization, to restore hybrid fertility by the creation of allopolyploids, to enhance pest resistance and disease tolerance in allopolyploids or to create larger plants with an enhanced vigor. In orchids has been reported that polyploid plants have larger flowers, show extended blooming time and develop flowers several times a year. Polyploids may be generated through the use of spindle formation inhibitors.

In this work we use colchicine and amiprophos-methyl [APM; O-methyl-O-(4-methyl-6-nitrophenyl)-N-isopropyl-phosphorothioamidate] as spindle inhibitors on colture of *Dendrobium* protocorm like bodies (PLBs). PLBs cultures were developed into liquid and solid medium and their ploidy was assessed in combination with the flow cytometric approach that allow us to do a fast and early screening of the treatment effects. We were able to identify which treatments were more effective and less harmful to our explants thus avoiding unnecessary mutagenic treatments and subculturing of unaffected materials .

We have also developed a protocol for the protoplasts isolation from PLBs with the final aim to isolate polyploid PLB protoplasts by means of flow sorting, This procedure allows us to recover a viable subpopulation of protoplasts enriched for higher ploidy level for further attempts on regeneration and whole plant recovery, thus reducing the chance for chimera formation.

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**Poster Communication Abstract – 9.12**

# WILD CARDOON VARIATION AND THE DOMESTICATION OF ARTICHOKE

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*Cynara cardunculus, genepools, SSR markers, domestication*

The genus *Cynara* L., *Asteraceae*, includes eight species and is distributed mainly in the Mediterranean regions where it forms a common and conspicuous component of the flora.

The cultivated globe artichoke (*C. cardunculus* var. *scolymus*) and the cultivated leafy cardoon (*C. cardunculus* var. *altilis*) belong to the same species, together with the wild cardoon (*C. cardunculus* var. *sylvestris*). The three *C. cardunculus* forms are fully cross-compatible with one another, and produce fertile hybrids. Many studies have confirmed wild cardoon to be the ancestor of both cultigens, which evolved independently under the influence of distinct anthropogenic selection criteria: globe artichoke for its capitula, and cardoon for its fleshy leaves and stalks.

The wild cardoon can be further subdivided into two types: the eastern and the western genepools, the former being mainly distributed in Italy, Tunisia, and Greece, while the latter diffused in Spain and Portugal. These two genepools can be distinguished for some morphological traits, e.g. the eastern plants are smaller and produce smaller flower heads with longer spines compared to wild cardoons from the Iberian peninsula. It has been hypothesized that the globe artichoke and the cultivated cardoon were domesticated from the eastern and western wild cardoon, respectively, in different times and places.

In this contribution, we have analysed a high number of Mediterranean wild cardoon populations, together with cultivated cardoon and artichoke materials, using SSR markers distributed over all the linkage groups of the *Cynara cardunculus* genome. Implications for the origin of the two cultigens are discussed.

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**Poster Communication Abstract – 9.13**

# IDENTIFICATION OF *HELIANTHUS* HYBRIDS THROUGH DNA BARCODES

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*Helianthus spp., interspecific hybrid verification, barcoding, ITS*

The nuclear internal transcribed spacer (ITS) sequence, bi-parentally inherited, is proposed as barcoding locus in plant kingdom. We evaluated the feasibility of ITS for following paternal and maternal genome lineages in interspecific hybrids in the *Helianthus* genus.

Seedlings of *Helianthus annuus*, *H. argophyllus*, *H. debilis* subsp. *cucumerifolius* and interspecific hybrids were cultivated at the experimental farm of Udine University (I). The interspecific hybrids were between *H. argophyllus* and *H. debilis* subsp. *cucumerifolius*, *H. debilis* subsp. *cucumerifolius* x *H. annuus* and *H. annuus* x *H. tuberosus*. Artificial hybrids were obtained by rubbing together the heads of parental species during the time of anthesis. The heads were bagged before anthesis and after pollination in order to prevent pollen contamination from other plants. Young leaves from each plant were collected for total genomic DNA extraction.

The species and hybrids were characterized at the molecular level through ITS. The nuclear regions were PCR amplified with specific primers and sequenced with an ABI Prism 3730 Automated DNA sequencer. Intraspecific and interspecific sequence variation was evaluated to assess the technique resolution. After sequence editing with specific software (Phred, Phrap and Consed) we was able to distinguish unambiguously each species looking for SNP.

In interspecific crosses, double peaks were clearly visible in those positions where the parental species were distinguishable enabling the correct identification of both parental species.

According to our results it is possible to discriminate the putative nature of hybrids between self- and cross-fertilization within *Helianthus* genus using ITS.

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**Poster Communication Abstract – 9.14**

# CYTOGENETIC CHARACTERIZATION OF CULTIVATED AND WILD SPECIES OF SUBFAMILY CICHORIOIDEAE (ASTERACEAE)

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*Molecular cytogenetics, rRNA genes, Lactuca, Cichorium, Chondrilla*

The subfamily Cichorioideae (Asteraceae) comprises several important genera which are widely represented in the cultivated and wild state. Among these *Lactuca* and *Cichorium* are economically important members since they contain many different varieties which are cultivated all around the world. Along with the cultivated forms there are a number of wild edible species which, since ancient times, have constituted an important popular food eaten fresh and boiled. Some of these species constitute the wild relatives or the direct ancestors of the cultivated forms. For this reason such large natural patrimony is regarded as an important source of genes to be used for the creation of improved new varieties. The present study has been carried out to increase the knowledge of *Lactuca* and *Cichorium* gene pools by means of a comparative cytogenetic analysis of cultivated and wild forms. To this end the karyomorphological analysis and the FISH mapping of ribosomal genes have been carried out.

In the last years particular attention has been directed to wild edible plants because of their remarkable content of nutrients and nutraceutical components. This is the case of *Chondrilla juncea*, a perennial plant with a distribution comprising Southern and Central Europe, North Africa, South Russia and Southwest Asia. In several countries, including Italy, it is an appreciated food used as salad separately or in combination with other wild greens. *C. juncea* is an obligate apomictic species in which diplosporous embryo sacs of the *Taraxacum* type are found. Presumably it is a triploid, its chromosome number is 2n=15. Owing to these features it represents an interesting case of study. Cytogenetic investigations were extended also to this species to verify the ploidy level and to observe the chromosome behaviour during microsporogenesis.

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**Poster Communication Abstract – 9.15**

# SOLIBAM: BREEDING BARLEY, BEAN AND BROCCOLI FOR ORGANIC AND LOW INPUT MANAGEMENT SYSTEMS

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*Low input, organic agriculture, breeding*

The SOLIBAM (*Strategies for Organic and Low-input Integrated Breeding and Managemen*) project, funded by the European Commission under the Seventh Framework Programme (GA 245058, coord. Véronique Chable, INRA, scientific responsible for UNIPG Valeria Negri) will develop specific and novel breeding approaches integrated with management practices to improve the sustainability, quality, performance and stability of crops and to develop varieties adapted to a wide range of organic and low-input management systems across several countries. A continuous release of new varieties of barley, bean and broccoli is foreseen among other project products. In Italy, barley (*Hordeum vulgare* L.), bean (*Phaseolus vulgaris* L.) and broccoli (*Brassica oleracea* L.) breeding has been carried out and the obtained lines/varieties are presently evaluated under two management systems (low input, LI, and organic agriculture, OA). In 2010, a barley composite cross originated population developed by UNIPG under zero nitrogen conditions was screened in Central Italy (Perugia) under OA and the best performing lines were selected. These have been evaluated under OA and LI in 2011 in comparison with other lines and cultivars. Seventeen lines of bean derived from crossing of an indeterminate growth habit landrace and a dwarf variety (coco nano) were tested under OA in Perugia (Central Italy) and LI in Grosseto (Tirrenic sea coast). Results obtained in the first year allowed to select two best performing lines in different management conditions which are being trialled under OA and LI in comparison with two control varieties. A phenotypic selection of a broccoli landrace was carried out and a 4 component (SYN_4C) and a 8 component (SYN_8C) synthetic populations were obtained. The two developed populations, the mother plant half sib progenies (HSMP) and two hybrid varieties were evaluated in 2011 under OA and LI. Obtained results show that the barley, bean and broccoli selected materials selected show good performances under OA and LI.

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**Poster Communication Abstract – 9.16**

# STRATEGY DEVELOPMENT TO IDENTIFY THE MOST APPROPRIATE AREAS FOR *IN SITU* CONSERVATION OF PLANT GENETIC RESOURCES

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*Biodiversity conservation, plant genetic resources, strategy development*

Plant genetic resources for food and agriculture are a subset of biodiversity and an essential basis for food security and human welfare. Protection of genetic resources *in situ* and access to a broad genetic variation are the cornerstones of a sustainable use of plant biodiversity in agricultural production systems.

Landraces (LRs) are traditionally grown crop distinct from modern varieties. Their traits can easily be introgressed into breeding pools. All the same crop wild relatives (CWRs) are a source of novel genetic variation and are increasingly used for crop improvement through breeding. Both LRs and CWRs are increasingly threatened with erosion or extinction by unsustainable agro-environmental management and ecosystem instability.

The *An integrated European in situ management workplan: implementing genetic reserves and on farm concepts (AEGRO)*' project (EC 057 AGRI GEN RES 870/2004 contract n. AGRI-2006-0396, http://aegro.jki.bund.de/aegro/, coor. L. Frese, J. Khun Institute, Quedlinburg, DE, scientific responsible for UNIPG, V. Negri) focused on the development of conservation strategies for both CWRs that occur in natural or semi-natural habitats and LRs that are found in farming systems. In the frame of this project, the aim of our work was to analyse the central Italy situation as a case study, and to develop and recommend efficient strategies to establish and implement LR conservation areas in EU member states.

As a first step, an inventory of LRs existing in central Italy has been created including data on LRs taxonomy and the biogeography of the cultivation sites. On the base of the collected data, LRs have been mapped by using orthophoto map and GIS program, in order to visualize and analyse the density and the distribution of LR cultivation areas.

Thereafter, criteria have been elaborated to be taken into account in delimitating areas which are the richest in LRs in order to restrict the number of sites to recommend for conservation activities with priority. The minimum number of criteria which allow the maximum inclusion of diversity has been identified. Two strategies aimed to detect the most appropriate areas (MAA) for the LR on farm conservation. MAAs are areas to be proposed to the National or Regional authorities as areas where to set or enhance political and economic actions in favour of LR and agrobiodiversity conservation with priority. The strategies are based on three criteria that reflect the attributes of agroecosystems: composition, structure and function. In the first strategy the criteria were applied in sequence, progressively selecting areas and finally identifying MAA. In the second strategy areas were scored for each attribute and scores summed to obtain a global index. The areas with the highest scores were considered MAA. The practical results obtained applying these

strategies to the case study (i.e. LRs in Central Italy) are compared. The developed strategies can be applied across all Europe.

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**Poster Communication Abstract – 9.17**

# COMMON BEAN DOMESTICATION IN MESOAMERICA AND ANDES HIGHLIGHTED BY NUCLEOTIDE DATA

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*Phaseolus vulgaris, crop evolution, nucleotide diversity, domestication*

Investigations into the evolutionary history of crop species is expected to highlight the structure and organisation of their genetic diversity and the role of the evolutionary forces that have been shaping this diversity. Such knowledge is a crucial prerequisite for efficient conservation and use of existing germplasm for the development of new improved plant varieties. The common bean (*Phaseolus vulgaris* L.) is a diploid (2n = 2x = 22) annual species that is predominantly self-pollinating and is the main grain legume for direct human consumption. It represents a rich source of protein, vitamins, minerals and fibre, especially for the poorer populations of Africa and Latin America. A particular evolutionary scenario has characterised this species: the existence of two main geographically distinct gene pools (Mesoamerica and Andes), where two independent domestication events occurred. Here, we analysed a wide collection of wild and domesticated *P. vulgaris* accessions (215) that represent a cross-section of the entire geographical distribution of the common bean, from northern Mexico to northern Argentina. Four gene fragments were sequenced, with the aim of the definition of the nucleotide diversity and the population structure that characterise the wild and domesticated populations from both the Mesoamerica and Andes gene pools. Moreover, the possibility of multiple or single domestication events were investigated, along with the identification of the putative geographical locations of these domestication events within each gene pool.

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**Poster Communication Abstract – 9.18**

# ASSESSMENT OF GENETIC VARIATION IN SICILIAN *HELICHRYSUM* (ASTERACEAE) AND IMPLICATION TO GERMPLASM CONSERVATION

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*AFLP, genetic variation, Sicilian Helichrysum, germplasm conservation, genebank*

*Helichrysum* Mill. (Asteraceae, Gnaphalieae) is distributed worldwide, and widespread in Europe and in the Mediterranean region. Since the classical antiquity it has been used as an ornamental plant, in pots and gardens. Today, *Helichrysum* is employed to produce dry flowers and as an ornamental plant resistant to drought. The genus is used in the pharmaceutical and cosmetic industries for its medicinal properties, namely anti-inflammatory, antiallergenic, antipsoriasis and diuretic, which are connected with its content of oils and flavonoids.

Most Helichrysum taxa are rare and vulnerable or at risk of extinction, so that their conservation requires an appropriate approach based on systematics and genetics. Genebank collections consist of multiple accessions of the same or similar taxa that make it possible to conserve a portion of the genetic variation. Therefore, it is necessary to assess the genetic variation of the taxa, and to identify which target alleles should be preserved. Besides the widely distributed alleles, priority should be given to local alleles, exclusive to populations in specific sites. Taxonomic misidentification of genebank accessions can be common, thus leading to confusion for germplasm users. Molecular markers can be used to solve this problem: they help to assess the relationships among *taxa*, and can provide species-specific diagnostic markers. For endangered species, a genetic analysis with the definition of a heterozygosity index at a high number of loci, has been used as a criterion to assess the fitness of populations. However, in the case of small wild samples, such as endangered species, it is difficult to determine if a population is in Hardy–Weinberg Equilibrium (HWE), and therefore to assess its heterozygosity correctly.

Alternatively, the polymorphism index gives a broad and general description of the genetic diversity within a sample. In particular, AFLPs (Amplified Fragment Length Polymorphisms), because of their high multiplexing degree, are very informative.

In this work, we describe the application of AFLP in an attempt to: *i*) investigate relationships between the rare and endangered Sicilian Helichrysum entities; *ii*) plan a strategy to capture the highest genetic variation that can be preserved in a genebank.

The results of the genotype analysis showed that the Helichrysum populations are poorly differentiated at the DNA level. The overall variation could be attributed to differences between individuals, rather than between populations.

The low genetic differentiation of *Helichrysum* populations suggests that the entities studied form a single group, probably derived from a common ancestor with high phenotypic plasticity for adaptation to different environmental conditions, and with consequent advantages for the survival

of the species. The genetic relationships are in accordance with the geographical distribution and solved some controversial points in their botanical classification.

The identification of local markers, were used to calculate the number of plants required to preserve a copy of each marker and therefore to plan a strategy for conservation of *taxa*.

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**Poster Communication Abstract – 9.19**

# CULTIVATION OF EVERGREEN AZALEA CUTTINGS IN NEUTRAL-ALKALI SOLUTIONS: A WAY TO SELECT NEW ORNAMENTALS

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*Abiotic stress, alkalization, rhododendron, pH, sodium bicarbonate*

Evergreen azaleas (*Rhododendron* spp.) are horticulturally important ornamental plants, which usually favour acid soil conditions, performing best when the pH is between 4.5 and 6.0. Often they show strong iron-deficiency chlorosis symptoms if growth on calcareous soil. This stress is one of the most serious difficulties in their cultivation. Only *R. ripense* was previously shown to tolerate alkali substrates.

In this study we performed a screening on 10 azaleas (*R.* 'Oomurasaki', *R. scabrum*, *R. macrosepalum* var. *hanaguruma*, *R.* 'Ryukyushibori', *R.* 'Juko', *R.* 'Shinsen', *R.* 'Susogo no ito' *R.* 'Fujimanyo', *R. tosaense* and *R. indicum*), looking for genetic resources tolerant to neutral-alkaline pH. Ten cuttings *per* genotypes were placed in three different solutions: the first (standard solution) contained deionized water and 0.5 g/L of Peter's fertilizer (20:20:20) with pH 6; in the second and the third solutions, 0.10 g/L and 1.00 g/L of anhydrous $NaHCO_3$ were added at the standard solution to adjust the pH to 7.5 and 9.0.

Every 7 days we evaluated: chlorophyll content (SPAD units), number of leaves open at the base, percentage of leaf damages (0=0%, 1=< 5%, 2=5-25%, 3=25-75% and 4=>75%), height, root size, and root quality, rated with ordinal classes from 0 to 4, to describe the ascending amount of biomass produced.

After 21 days, a statistical effect of pH on cutting development was highlighted. Solutions with pH 7.5 and 9.0 induced the highest decrease of chlorophyll content. However, among genotypes, *R. scabrum*, *R.* 'Juko', and *R. macrosepalum* var. *hanaguruma* showed a low loss (-1.26, -2.03 and -4.06 SPAD units, respectively). Similar results were obtained for ornamental characteristics. Neutral-alkali solutions negatively affected azalea quality but again, among genotypes, *R. macrosepalum* var. *hanaguruma* and *R. scabrum* presented the lowest leaf damages (0.33 and 0.61, respectively) and the highest root production (3.00 cm and 1.41 cm, respectively). Therefore, these two species showed a potential neutral-alkali tolerance. By contrast, at the same time point, *R.* 'Shinsen' and *R. indicum* cuttings showed a percentage of leaf damages over 75% (3.33 and 3.46, respectively), presenting chlorosis, leaf abscission, and roots browning.

In conclusion, the present work confirmed a significant effect of pH on azalea quality and development. Among the 10 tested genotypes, *R. scabrum* and *R. macrosepalum* var. *hanaguruma* revealed a particular tolerance to neutral-alkali pH. These species are taxonomically grouped in the Subsection *Macrosepala* together with *R. ripense*, whose tolerance to neutral-alkaline pH was already known. To better understand the molecular bases of this stress tolerance, molecular analyses by means of STMS markers are in progress.

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**Poster Communication Abstract – 9.20**

# MOLECULAR AND CHEMICAL MARKERS TO TRACE THE GENETIC IDENTITY AND THE GEOGRAPHICAL ORIGIN OF POTATOES

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*Molecular markers, traceability, authentication, multi-elements, Sr isotope ratio*

There is a growing interest in agricultural productions combining safety and quality attributes with clear regional identity. Therefore, in the last few years several strategies have been employed to refine the capacity for the authentication of food products. In the frame of a project funded by MiPAAF, we emploied SSR markers to distinguish the potato varieties commonly used in southern Italy for early productions. In addition, we also applied multi-element and Sr-isotope ratio analyses to determine their geographical origin. SSR analysis allowed the identification of 37 alleles analysing 8 microsatellite loci. Only two alleles were present in all varieties, while the other 35 showed a varying degree of polymorphism. The presence of private alleles was highlighted in all the varieties, allowing the identification of each of them. The elemental concentrations investigated were [Mn], [Cu], [Zn], [Rb], [Sr] and [Cd]. They were combined to the Sr isotopic signature data. Results provided evidence that potatoes grown in soils formed from alluvial sediments were characterised by lower amounts of Rb, those grown in soils formed on carbonate rocks showed higher Cd contents, while potatoes grown in soils formed from volcanic substrates have higher amounts of Zn and lower $n(^{87}Sr)/n(^{86}Sr)$. The combined use of biological and chemical markers to prevent frauds and to valorize food products is discussed.

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**Poster Communication Abstract – 9.21**

# TRACING THE BIOLOGICAL ORIGIN OF SICCATIVE OILS USED IN PAINTINGS THROUGH CHLOROPLAST DNA ANALYSIS

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*Ancient DNA, oil DNA, chloroplast DNA analysis, DNA barcoding*

In order to realize their paintings, Renaissance artists commonly used a variety of natural binding media selected on the basis of technological and esthetic criteria. The characterization of binders provides valuable information for art historians and conservators toward a better understanding of painting techniques and in planning the best conservation strategies. In fact, this would be very helpful in determining their authenticity, as well as revealing important historical, economic, social, and cultural aspects. The characterization of binding media is currently carried out on micro-samples taken from the cultural heritage object and analyzed by vibrational spectroscopy and gas-chromatographic methods. Micro-FTIR analysis provides valuable chemical composition of the binder, allowing for a first discrimination between protein, glycosides, and lipids, while GC-MS gives insight on their specific typology, but does not allow identifying their biological origin. In this context, we moved toward the exploitation of the high specificity and high sensitivity offered by the state-of-the art DNA analysis, focusing our efforts in the development of a suitable protocol for the identification of the biological origin of binding media on minuscule samples obtained from ancient paintings. We have already applied this strategy for the molecular characterization of mitochondrial regions of species traditionally employed in animal-based glues (Albertini et al. 2011, Anal. Bioanal. Chem. 399:2987-2995). We now report the development of a suitable protocol for the identification of the biological origin of oil binding media found in tiny samples obtained from ancient paintings through DNA analysis. The current protocol discussed in this study was developed using fresh siccative oils (flax, poppy, and walnut oils) from aged painting models and old painting samples.

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**Poster Communication Abstract – 9.22**

# VALIDATION OF GENES INVOLVED IN STRIGOLACTONES BIOSYNTHETIC PATHWAY IN *LOTUS JAPONICUS*

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*Strigolactones, plant branching, biosynthetic genes, Lotus japonicus, stress conditions*

As sessile organisms, plants elaborate their development of branches in response to environmental and developmental signals to maximize reproductive success. Hormones play a critical role in determining the diversity of plant branching. One of the signaling pathways regulating branching involves MAX/RMS/D (more axillary branching) genes, which are apparently highly conserved in higher plants. Strigolactones, a group of terpenoid lactones, have been recently identified as products of the MAX/RMS/D pathway that travel acropetally from root through xylem to inhibit bud outgrowth. Here we report the cloning of the first catalytic enzyme for strigolactones (*CCD7*) from *Lotus japonicus* (*LjCCD7*) by RACE-PCR. *LjCCD7* contains a 1866bp ORF encoding 621 amino acids, and is most closely related phylogentically to *RMS5* of pea, *CCD7* of soybean, Arabidopsis and tomato. *LjCCD7* was then prokaryotically expressed as GST fusion protein, and detected by both SDS-PAGE and western blot analysis. As in other species, enzymatic activity of the purified GST-LjCCD7 indicated that it encodes an enzyme capable of cleaving carotenoids such as beta-carotene. Plants silenced for *LjCCD7* were generated, and are being molecularly characterized. Their phenotype is currently being analyzed under different stress conditions.

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**Poster Communication Abstract – 9.23**

# ADAPTATION OF VETCH, FIELD BEAN AND LUCERNE TO ORGANIC FARMING

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*Vicia sativa, Vicia faba, Medicago sativa, organic farming, GxE interaction, multilocation trials*

The most important results found in vetch, field bean and lucerne are reported and referred to the first year of research activities. In field bean eight varieties (Chiaro di Torre Lama, Irena, Melodi, Prothabat 69, Scuro di Torre Lama, Sicania, Sikelia e Vesuvio) were evaluated in five locations (Ancona, Perugia, Grosseto, Bari e Catania, a good sample of environments where field bean is grown). Statistically significant differences were found for most of traits: establishment, plant density, cold tolerance, flowering time, canopy height, disease resistance, grain yield and 1000-seed-weight. Significant differences among Location were shown for seed yields, with Bari being the highest (3.3 t/ha) yielding site. On the base of the first year results Prothabat 69 was the most interesting variety in terms of seed yield and seed weight, while at the same time Melodi ranked almost always last.

The eight varieties of vetch (Adonis, Ereica, Melissa, Mikaela, Mirabella, Principessa, Sfinge e Veronica) were evaluated in the same locations as those listed for field bean and, in addition to the same traits already described, in vetch the dry matter yield was measured in half of the plot, by harvesting the biomass present at full flowering time, while seed yield was assessed on the remaining half, at maturity. Differences were found for the source of variation Locations and Variety. The most interesting vetch varieties in terms of dry matter yield were Ereica and Mirabella. A significant GxE interaction showed different adaptation capability of the varieties in the five locations: Principessa for instance was the best in Catania, the most unfavourable environment, while Mirabella and Ereica were the best in the most favourable ones. Melissa was of no particular interest.

The results of lucerne are preliminary being referred to the establishing year. However, at this stage Cuore Verde, Beatrix and Prosementi seem to be the most interesting cultivars in terms of dry matter yield.

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**Poster Communication Abstract – 9.24**

# EPIGENETICS ASPECTS OF POLYPLOIDIZATION: STUDIES OF EXPRESSION PATTERN OF DNA METHYLATION GENES IN *MEDICAGO SATIVA* L.

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*Alfalfa, epigenetics, polyploidization, DNA methyltransferase genes*

The widespread occurrence of epigenetic alterations as a consequence of polyploidization in plants indicates that DNA methylation systems may be perturbed by polyploidy changes. DNA methylation genes are involved in many vital developmental and physiological processes of plants.

In this work, we seek novel information on epigenetic consequences of autopolyploidization in alfalfa (*Medicago sativa*). In particular, genes responsible for the DNA methylation status are studied in 2x, 3x and 4x genotypes progenies obtained by crossing two 2x plants that produce both n and 2n eggs and pollen, respectively. To identify DNA methyltransferase genes and their expression patterns we examined some of the elements of three major DNA methyltransferase families MET1, CMT and DRM. Methylation at CG nucleotides is maintained in plants by the the enzymes of the MET1 family typical of higher eukariotes. The second family, called Domain Rearranged Methyltransferase (DRM) has characteristic rearrangement of conserved motifs in the catalytic domain and probably catalyzes methylation of native DNA. The chromomethylases (CMT) are unique of higher plants. These enzymes maintain methylation of CHG trinucleotides.

*In silico* searches have lead to the identification of *M. sativa* methyltransferase genes homologous to known plant methyltransferase genes. Oligonucleotides have been designed from these sequences in order to analize the expression pattern of these genes. Gene expression changes induced by polyploidization are being investigated using qRT-PCR and expression data will be validated and jointly analyzed to identify ploidy-affected genes.

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**Poster Communication Abstract – 9.25**

# MOLECULAR ANALYSIS OF T-DNA INSERTION EVENTS IN ALFAFA

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*T-DNA, alfalfa, TAIL-PCR*

*Agrobacterium tumefaciens*-mediated transformation is an established method to introduce foreign genes into plants. This bacterium is capable of transferring a DNA (T-DNA) comprised between two imperfect border repeats (left border, LB and right border, RB). Anomalous T-DNA production, random insertion into the plant genome and possible DNA rearrangements during T-DNA integration make each transformation event unique and not reproducible.

Alfalfa is an important forage crop in which *Agrobaterium*-mediated transformation is established, but no information is reported in literature about the characterization of integration events.

Using TAIL-PCR, we cloned the T-DNA integration sites of some transgenic lines produced in our laboratory . Preliminary results indicate that TAIL-PCR can be successfully used to isolate junction sequences between the T-DNA borders and the alfalfa genome. Sequence alignment reveals features of T-DNA processing at LB by *Agrobacterium*. BLAST analyses of the insertion site sequences reveals homologies with the genome of *Medicago truncatula* (L.), a closely related species. Further analyses of LB and at RB junction sequences are in progress and will be presented.

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**Poster Communication Abstract – 9.26**

# MANAGING GENETIC DIVERSITY IN THE CONSTRUCTION OF ALFALFA SEMI-HYBRIDS

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*Alfalfa, genetic diversity, heterosis, SSR markers*

The construction of narrow genetic based varieties – and in particular semi-hybrids – in alfalfa asks for an effective handling of genetic diversity. A part of genetic diversity in this forage crop is in relation to subspecies subdivision (*falcata*/*sativa*); non dormant germplasm from Sahara oases could represent a putative 'pure' cultivated ssp. *sativa* and its crossing with the european germplasm is likely to express heterotic effects. In order to test this hypothesis, $S_2$ parental families selected for vigour in four Italian ecotypes and in four landraces collected in the Siwa oasis (Egypt) were crossed to produce 58 simple hybrids (SH) $S_2xS_2$: 13 Egyptian x Egyptian (EE), 33 Egyptian x Italian (EI) and 12 Italian x Italian (II). The bio-agronomic test of SHs (in general 80 plants/SH; 6400 plants in total) was carried in outdoor condition in microplots 25x80 cm (20 plants/plot) with density equivalent to 400 plants.$m^{-2}$. The data of the sowing year 2010 (5 harvests) are reported.

The $S_2$ parental families (1-9 plants/family, in bulk) were analysed by means of 68 SSR markers: the average diversity among the two germplasm sources Egyptian and Italian was 0.4723, while within-germplasm diversity resulted 0.4178 and 0.4698 respectively for the Egyptian and Italian source. The absence of a clear subdivision between the two germplasm sources was confirmed by UPGMA tree. On the contrary, it was evident an important increase in the among-families diversity following the two generations of selfing and selection: in fact the distance, estimated on a subset of 31 SSR markers, among the original four Egyptian landraces was 0.0326 compared to the value of 0.4585 for the derived $S_2$ parental families.

Dry matter yield (DMY) of the three groups of SHs (EE, EI, II) was not significantly different except for the 5$^{th}$ harvest (October) in which the EI group produced more than EE and II. A subset of 15 SHs derived from the diallel crossing of three Egyptian and three Italian $S_2$ families was examined by diallelic analysis. General combining ability (GCA) was highly significant and consistent through harvests for DMY, stem height and earliness, while specific combining ability (SCA) was never significant except in the 5$^{th}$ harvest for DMY and stem height. The parental $S_2$ family derived fron the Italian ecotype 'Friulana Premariacco' showed significant and positive GCA effect for DMY and earliness, while a family derived from Siwa landrace 13 for stem height.

A clear heterotic pattern between the two germplam sources (Egyptian and Italian) was not put in evidence. On the contrary, the crossing of $S_2$ families with positive GCA effects from the two germplasm sources seems suitable to effectively combine features implied in vigour (persistency, DMY, stem elongation rate).

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**Poster Communication Abstract – 9.27**

# CHARACTERIZATION OF SOME *P. VULGARIS* AND *P. COCCINEUS* CULTIVARS FROM GARFAGNANA REGION IN TUSCANY BY TRAP MOLECULAR MARKERS

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*Common bean, TRAP molecular markers, genetic variability analysis*

There has been a long, widespread tradition of common bean cultivation in Italy since it was introduced from the Americas. Up to now, selection by farmers has led to the production of a number of cultivars adapted to the new and heterogeneous environments in several Italian regions. In particular, in Tuscany, the genus *Phaseolus* has acquired an important role in the traditional diets, so that Tuscany has become a region with a great variety of landraces. Moreover, some landraces are typical of sub-regional restricted growing areas, where several genotypes have been selected and maintained through the adaptation to the specific pedo-climatic conditions and the traditional agro-techniques. Such landraces have often acquired, highly desirable quality traits through man-driven selection for plant habit, seed color, seed pattern type and also disease and pest resistance, and are among some of the most appreciated Italian common bean cultivars. This is the case, for instance of the Garfagnana area, in Tuscany, where autochthonous cultivars, highly valuable, though with rather low productivity are grown mainly with traditional methods in kitchen-gardens, small-holders' family farms and in dwelling areas, either for personal consumption or for sale as specialties in farmer markets. Nowadays, apart from some traditional cultivars which have acquired national or European quality and origin marks, common bean is mostly cultivated in intensive agricultural systems aiming to assure a good production, in accordance with market demands.

As a matter of fact, old varieties are gradually being replaced by improved cultivars, and the remaining ones are confined to marginal areas, so that most of them are likely to be endangered due to the low amount of seed per plants and to the socio-cultural context where they are cultivated.

A way to safeguard this autochthonous germplasm is to deepen the knowledge of their genetic, morphological and agronomical characteristics. So, the aim of this study has been the molecular characterization of local cultivars of the genus *Phaseolus*, maintained in the germplasm bank collection of the "Comunità Montana della Garfagnana", where farmers have actually practiced the *on farm* maintenance of such genetic resources. Target region amplification polymorphism (TRAP) markers were used to assess genetic variability among 12 germplasm accessions of *Phaseolus vulgaris* and *Phaseolus coccineus* from the Garfagnana area. Commercial varieties of the genus *Phaseolus* were also examined as an out-group. TRAP markers were generated from four fluorescent labeled arbitrary primers in combination either with two fixed primers derived from the *Arabidopsis*-like telomere repeat sequence or with two fixed primers designed against exons 1 and 2 of *Phaseolus* CHS gene family. A relatively high level of polymorphism was found within accessions; similarity index and the following cluster analyses showed that all populations clustered into two groups corresponding to the *P. vulgaris* and *P. coccineus* and that the commercial varieties grouped separately from the landraces. Some

preliminary considerations about the correlation between TRAP dendrogram clustering and seed morphological aspects were drawn.

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**Poster Communication Abstract – 9.28**

# CHROMOSOME PAIRING BEHAVIOUR IN NEWLY SYNTHESIZED TETRAPLOID ALFALFA

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*Polyploydy, alfalfa, chromosome pairing, sexual polyploidization*

Polyploidization is an increase in genome number and occurs in nature as the consequence of the union of gametes with the somatic chromosome number 2n (sexual polyploidization), or as the consequence of somatic genome duplications (somatic polyploidization). A duplication of a species' chromosomes results in the formation of a polyploid with polysomic inheritance, while the union of the genomes of different species results in the formation of a polyploid with disomic inheritance. Some polyploids have both modes of inheritance and are termed segmental allopolyploids.

We are studying three tetraploid plants from bilateral sexual polyploidization (BSP) obtained by crossing a diploid *Medicago sativa* subsp. *falcata* plant that produces 2n eggs (PGF9) with a 2x *Medicago sativa*. subsp. *coerulea* x *falcata* plant that produces 2n pollen (12P). Each of these BSP plants was crossed as female with a plant from the tetraploid cultivated variety *Classe,* and 50 progeny plants were obtained per cross. Cultivated alfalfa has tetrasomic inheritance; however, the newly tetraploidized BSP plants used in this work derive from a cross between morphologically and genetically different diploid parents and therefore it is possible that differences in chromosome structure lead to preferential pairing between two of the homologous chromosomes from the same parent. To investigate chromosome pairing behavior of the tetraploidized BSP plants segregation of polymorphic SSR markers are emploied. One marker per chromosome was selected from the published resources based on their usefulness in identifying suitable polymorphisms among the parents and progeny plants. The observed SSR segregation patterns will be compared with those expected in case of disomic (preferential pairing) or tetrasomic (random pairing) inheritance.

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**Poster Communication Abstract – 9.29**

# THE EFFECTS OF DOMESTICATION ON THE STRUCTURE OF THE NUCLEOTIDE DIVERSITY IN THE COMMON BEAN FROM MESOAMERICA

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*Phaseolus vulgaris, crop evolution, nucleotide diversity, selection, linkage disequilibrium*

The common bean (*Phaseolus vulgaris* L.) is a diploid (2n = 2x = 22) annual species that is predominantly self-pollinating and is the main grain legume for direct human consumption. The species is characterised by two major geographically distinct gene pools that predate its domestication, and where two independent domestication events occurred: Mesoamerica and the Andes. Many studies have investigated the molecular and phenotypic diversities and the population structure of the common bean, although little information is available on the level and extent of its nucleotide diversity. Here, we focused our attention on investigation of the domestication process in Mesoamerica by sequencing 55 gene fragments from a set of 47 accessions of common bean, most of which were from Mesoamerica (39; as both wild and domesticated forms). Eight additional accessions were included as controls: four from the Andes, two from northern Peru–Ecuador wild populations that are characterised by phaseolin type I (a seed storage protein), and one as a *P.coccineus* and *P. dumosus* accession. Nucleotide diversity, population structure and linkage disequilibrium analyses were carried out, along with identification of loci that show signs of a past genetic sweep, which are indicative of selection during domestication.

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**Poster Communication Abstract – 9.30**

# CYTOKININS-AUXIN DEPENDENT MOLECULAR MECHANISMS NECESSARY FOR THE STEM CELL NICHE MAINTENANCE IN THE *ARABIDOPSIS THALIANA* ROOT MERISTEM

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*Root Meristem Mainteinance, Stem Cell Niche, Cytokinin-Auxin crosstalk, Scarecrow*

Understanding the molecular mechanisms through which plant meristems are maintained is a central question in developmental biology. In the root of *Arabidopsis thaliana*, stem cells in the apical region of the meristem self-renew and produce daughter cells that differentiate in the distal meristem transition zone. To ensure root growth, the rate of cell differentiation must equal the rate of generation of new cells. Cell differentiation takes place in the transition zone that is localized in the distal part of the root meristem, but must be synchronized and balanced with division of the stem cells that are localized in the apical part of the meristem. We have previously shown that maintenance of the *Arabidopsis* root meristem size - and consequently root growth - is controlled by the interaction between two hormones at the meristem transition zone: cytokinins, which promote cell differentiation, and auxin, which promotes cell division, but it is still unknown how the cytokinin/auxin interaction maintains a balance between cell differentiation at the transition zone and cell division in the stem cell niche. Here we show that SCARECROW (SCR) maintains stem cell activity repressing cytokinin-mediated differentiation input in the stem cell niche through down-regulation of the cytokinin-responsive transcriptional regulator ARR1 thus controlling root meristem size.

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**Poster Communication Abstract – 9.31**

# NON-TOXIC MUTANT FORM OF SAPORIN FOR DEVELOPING CANCER VACCINES

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*Ribosome Inactivating Proteins, Saporin, HPV vaccination, E7 protein*

Saporin is a type 1 (single chain ) ribosome-inactivating protein (RIP) present in different organs of the soapwort (*Saponaria officinalis* L., Caryophyllaceae). RIPs are potent inhibitors of protein synthesis, enzymatically removing a specific adenine residue present in a conserved stem and loop region of the 23S/25S/28S rRNA (ricin-sarcin loop).

Although saporin cytotoxicity, due to protein synthesis arrest, was the first feature to be characterized and clinically exploited, other biological activities exist (i.e. immunogenicity, ability to modulate immune functions and apoptosis induction) that could be useful tools in tumour immunotherapy.

We have investigated the potential of saporin as an innovative immuno-stimulatory carrier, able to increase immune 'visibility' of a weak antigens. A non toxic mutant form of saporin (SAP-KQ) was used as a carrier for the E7GGG gene, an attenuated form of the high risk HPV type 16 (Human Papilloma Virus) gene coding for E7 oncoprotein.

We show that fusion constructs of SAP-KQ with E7GGG can induce E7-specific Immunoglobulins (IgGs), Cytotoxic T Linphocytes (CTLs) and Delayed-Type Hypersensitivity (DTH) affecting the growth of E7-expressing tumors in mice.

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**Poster Communication Abstract – 9.32**

# IS THE REDOX ACTIVITY OF PLASMA MEMBRANE CYTOCHROME AIR12 INVOLVED IN CELL SEPARATION EVENTS?

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*Redox, plasma membrane, cell wall, auxin*

AIR12 is the major plasma membrane cytochrome b belonging to a new family of ascorbate-reducible cytochromes b specific to flowering plants. Based on GFP-localization experiments and sequence analysis, AIR12 is suggested to be bound *in vivo* to the external side of the plasma membrane by means of a GPI-anchor. Moreover, AIR12 has been found associated with lipid rafts both in Medicago and Arabidopsis.

Arabidopsis AIR12 was heterologously expressed in *Pichia pastoris* and shown to be a high-potential cytochrome b with a symmetrical $\alpha$-band at 560 nm. Ascorbate, superoxide and nafto-hydroquinones are all potential reductants of AIR12, whereas monodehydroascorbate works as potential electron acceptors. Although oxygen is not an efficient electron acceptor for AIR12, the *in vitro* characterization of AIR12 is hindered by the rapid oxidation of reduced AIR12 in the presence of oxygen.

Purified and permeabilized plasma membranes vesicles additioned with NADH, menadione e and FeEDTA produce superoxide and hydroxyl radicals that can be detected by EPR. We have found that further addition of recombinant AIR12 causes an increase in the production of both radicals, suggesting a pro-oxidant role of the protein under these conditions.

Arabidopsis lines transformed with the AIR12 promoter fused to the GUS or GFP genes show localized expression at sites where cell separation events occur (e.g. lateral root caps, root epidermis at site of lateral root emergence, micropylar endosperm during germination, anthers and floral organ abscission zones, hydatodes) as well as in the vascular bundles of mature leaves and trichomes support cells. Exogenously applied auxins boost reporter gene expression in the entire roots while ABA enhances expression specifically at the root tip. From available data, a role of AIR12 in cell wall modification processes is proposed, possibly involving its pro-oxidant activity.

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**Poster Communication Abstract – 9.33**

# CALCIUM INFLUXES AND PROTEIN KINASE ACTIVATION MEDIATE OZONE-INDUCED DEFENCE GENE EXPRESSION IN TOBACCO PLANTS

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*Calcium, defence genes, MAPKs, nitric oxide, ozone*

Ozone ($O_3$) can affect several processes in plants including the transcription of many defence associated genes, however, the mechanism by which $O_3$ brings about these changes remains largely unknown. We have shown that nitric oxide (NO) is a second messenger in the signalling cascade induced by $O_3$ leading to defence gene induction. The NO action is accomplished by both cGMP dependent and cGMP-independent pathways; in particular, the activation of *AOX1a* is independent from cGMP. By contrast, the early response of phenylalanine ammonia lyase (*PALa*) and the late response of pathogenesis related protein (*PR1a*) show critical dependence on cGMP. This research is aimed to unravel the role that calcium and protein kinases play in the transduction pathway leading to defence gene activation. $O_3$ induces rapid activation of a MAP kinase of approximately 48 kDa which by the immune-complex kinase activity assay was identified as salicylic acid induced protein kinase (SIPK). The SIPK activation was transient and the activity returned to basal level within 3 h of $O_3$-fumigation. When the $O_3$-induced NO accumulation was reverted by the NO quencher cPTIO no SIPK activation was detected in tobacco ozonated plants, suggesting that NO acts upstream of MAPK cascade. However, when we inhibited SIPK activity by the Ser/Thr kinase inhibitor staurosporine (Stau), we found a complete reversion of the $O_3$-induced *AOX1a* and *PALa* activation, and this suggests a central role of the protein kinase mediated phosphorylation in the $O_3$-induced up-regulation of these genes. For *PR1*a the picture is more complex. In tobacco plants challenged with $O_3$ *PR1a* was found to be up-regulated after 24 h; the application of Stau markedly induced *PR1*a mRNA accumulation in the absence of $O_3$, suggesting that a protein dephosphorylation mediates the *PR1a* expression in tobacco. An addictive effect on *PR1a* mRNA accumulation was found when both stimuli (Stau + $O_3$) were applied. We conclude that all the examined genes (*AOX1a*, *PALa* and *PR1a*) are dependent on protein kinases for their expression. Calcium has been demonstrated to be an important molecule that mediates signal transduction. To elucidate whether $Ca^{2+}$ is involved in $O_3$-induction of target genes, cytosolic $Ca^{2+}$ influx was inhibited by lanthanum chloride ($LaCl_3$) and ruthenium red (RR). Lanthanum chloride is known to compete externally with $Ca^{2+}$ for channels located in the plasma membrane, while RR blocks the ryanodine ion channels that control $Ca^{2+}$ mobilization from internal stores in both animals and plants. The *AOX1a* was transiently induced by $O_3$ with highest mRNA accumulation between 2 and 5 h of $O_3$ fumigation; the application of both RR and $LaCl_3$ did not change its expression profile, suggesting that *AOX1a* gene expression was not influenced by $Ca^{2+}$. A different picture was obtained for *PALa* expression which was induced during $O_3$-fumigation, but its activation was completely suppressed by RR, whereas was not influenced by $LaCl_3$. The pattern of *PR1a* expression under $O_3$, similarly to *AOX1a*, shows that the expression of this gene was independent

on $Ca^{2+}$, because neither RR and $LaCl_3$ did not suppress $O_3$-dependent induction. A working model showing how $Ca^{2+}$ and protein kinases cross talk with NO-mediated transduction pathway in the induction of defence genes will be presented.

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**Poster Communication Abstract – 9.34**

# OZONE EFFECT ON RAGWEED POLLEN VIABILITY AND NAD(P)H OXIDASE ACTIVITY

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*NAD(P)H oxidase, ozone, pollen, ragweed, ROS*

*Background*

The increase in respiratory diseases arising from allergies in industrialised countries in recent years is considered to be linked to changes in certain environmental factors. One such factor relates to the higher levels of atmospheric pollution and the greater presence and distribution of allergenic *taxa.* We investigated the effects of ozone ($O_3$), the main component of the photochemical smog, by exposing ragweed (*Ambrosia artemisiifolia*) pollen to a high $O_3$ concentration (100 nL $L^{-1}$) for 7 days. We specifically evaluated: pollen reactive oxygen species (ROS) and nitric oxide (NO) content, as NO is considered to be a mediator of inflammatory responses; activity of the nicotinamide adenine dinucleotide phosphate (NAD[P]H) oxidase, which can generate ROS; expression of the major ragweed pollen allergens; and pollen viability.

*Results*

We investigated the impact of $O_3$ on ROS and allergen content of ragweed pollen. Pollen was exposed to acute $O_3$ fumigation, with analysis of pollen viability, ROS and NO content, activity of NAD(P)H oxidase, and expression of major allergens. There was decreased pollen viability after $O_3$ fumigation, which indicates damage to the pollen membrane system, although the ROS and NO contents were not changed or were only slightly induced, respectively. Ozone exposure induced a significant enhancement of the ROS-generating enzyme NAD(P)H oxidase. The major allergenic proteins released from ragweed pollen ranged from 8 kDa to 43 kDa, with similar protein pattern profiles in both control and $O_3$-fumigated pollen. The major antigenic component of ragweed pollen, Amb a 1, was identified as a band visible at about 38 kDa, and Western blotting analysis revealed that its content did not change after $O_3$ exposure. We also examined the expression profiles of the major ragweed pollen allergens: Amb a 1 and Amb a 2, which are proteins belonging to the pectate lyase family, and profilin 1 and profilin 2, which are proteins involved in signal transduction from the outer cell membrane to the inside of the cell, and in the regulation of actin polymerisation. Our data show that $O_3$ exposure did not affect the expression of the major ragweed pollen allergens.

*Conclusions*

Pollen represents a critical stage in the life cycle of plants, as viable pollen is crucial for efficient sexual reproduction in plants. Our data indicate that exposition of ragweed pollen to realistic $O_3$ concentrations reduces pollen viability. As the pollen viability is significantly related to pollen germination and the length of the pollen tubes, effects on the reproduction of ragweed in polluted situations should be taken into account. The present study also indicates that there is an impact of the air pollutant $O_3$ on the ROS/NO content, NAD(P)H oxidase activity and allergens of ragweed pollen grains. While the intrinsic ROS and allergen content were not affected by $O_3$ fumigation, there was significant enhancement of the activity of the ROS-generating enzyme NAD(P)H oxidase. This enzyme was almost completely released after short times of *in-vitro* pollen

hydration, as occurs in nature when pollen comes into contact with the cells of the respiratory apparatus. We conclude that realistic doses of $O_3$ can increase ragweed pollen allergenicity through stimulation of NAD(P)H-oxidase-mediated ROS generation at the airways level.

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**Poster Communication Abstract – 9.35**

# PROTEOMIC PROFILING OF WHITE TRUFFLE (*TUBER MAGNATUM* PICO) NATURALLY GROWN IN DIFFERENT ITALIAN AREAS

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*Proteomic, truffle, two-dimensional electrophoresis, mass spectrometry*

The aim of this work is to verify the origin of the white truffle (*Tuber magnatum* Pico) through protein profiling in which it would be possible to identify one or more proteins related to the area of origin. Truffles are considered an important food resource with a high economic value as high as 300 euros/100 gr. of fresh product. The *Tuber magnatum* variety is particularly appreciated and its demand often outweighs the amount of truffles harvested in its natural habitat.

This species grows almost exclusively in Italy, and its value is related to the area of collection (North, Center or South Italy).

Therefore, it would be crucial to set up a methodology capable of discriminating univocally not only the species analysed, but also its origin, in order to distinguish the more valuable varieties from the less expensive ones.

In order to achieve this goal, samples of *Tuber magnatum* Pico, from selected areas in Tuscany, Piedmont, Umbria and Marches, were collected and then analysed through a proteomic approach. The analyses performed so far using bidimensional electrophoresis have shown a high reproducibility in the protein pattern of *Tuber*.

The preliminary analysis of differentially expressed proteins, by using LC-MS spectrometer, has allowed us to identify specific proteins.

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**Poster Communication Abstract – 9.36**

# PROTEOMIC ANALYSIS OF COLD STRESSED *ARABIDOPSIS THALIANA* CHLOROPLASTS

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*Cold stress, chloroplasts, two-dimensional electrophoresis, mass spectrometry*

Low temperature is one of the major abiotic stresses limiting the productivity and the geographical distribution of many species. The effect of cold acclimatization is evident at chloroplast level, for this reason our aim is to analyze the change in level of expression of chloroplast proteins during stress. 13-days old plants were acclimatized at 4°C for 1 week and treated at -10°C for 12 h and then recovered for 24 h. Freezing treatment produced stress phenotypes of rolling leaves, increase in electrolyte leakage and decrease in pigment content. The changes of total proteins in chloroplasts were examined using two-dimensional electrophoresis. Among 200 protein spots reproducibly detected on each gel, we found up- and down-regulated spots. Mass spectrometry analysis allowed the identification of 30 differentially expressed proteins, including well know cold-responsive proteins. Several proteins showed enhanced degradation during freezing stress, especially the photosynthetic proteins such as Rubisco activase (RcbA) and Rubisco large subunit (RcbL) of which 4 fragments were detected. The identified proteins are involved in several process: photosynthesis, RNA processing, protein translation and processing, metabolism of carbon, nitrogen end energy. These proteins might work cooperatively to reach an homeostatic equilibrium to overcome stress conditions.

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**Poster Communication Abstract – 9.37**

# CRYSTALLIZATION OF PHOTOSYSTEM II FROM *N. TABACUM*

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*Photosystem II core complex, Higher plants, Nicotiana tabacum, 3D crystallization*

Water-splitting photosynthesis also known as oxygenic photosynthesis is a process whereby light energy is converted to chemical energy in membrane-bound pigment protein complexes located in the thylakoid membranes of higher plants and algae. Photosystem II (PSII) is one of the membrane protein complex involved in the photosynthetic process and it is characterized by a dual function: the capture of the light and the splitting of the water molecule. More in detail, PSII uses the electrons delivered during the water splitting for storing in a form of chemical energy the light energy absorbed from the protein complexes associated pigments. As a secondary product the atomic oxygen, produced from the splitting of the water, is converted to molecular oxygen and delivered to the atmosphere. Several structural and functional features of this pigment protein complex from higher plants are not completely understood. In order to address several questions, related with the function and the structure of PSII from higher plants, a fast and stable protocol of purification was developed and also a protocol of crystallization of PSII has been created. At moment the quality of the crystals needs to be improved in order to get them suitable for definitive structural and functional studies.

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**Poster Communication Abstract – 9.38**

# GLUTAREDOXIN S12: UNIQUE PROPERTIES FOR REDOX SIGNALING

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*Cysteine, oxidation, glutathionylation, glutaredoxin, redox sensor*

Cysteines made acidic by the protein environment are generally sensitive to pro-oxidant molecules. Glutathionylation is a post-translational modification which can occur either by GSSG-mediated thiol/disulfide exchange or by the reaction of reduced glutathione (GSH) with oxidized cysteines as sulfenic acids (-SOH). The reverse reaction (deglutathionylation) is strongly stimulated by small disulfide oxidoreductases named glutaredoxins (Grx) that require glutathione (GSH) or thioredoxin reductases for their regeneration.

By using structural analyses coupled with thiol titrations, fluorescence measurements, site-directed mutagenesis and mass spectrometry we have determined the structural and biochemical properties of poplar GrxS12, an atypical chloroplastic Grx possessing a monothiol active site ($_{28}$WCSYS$_{32}$). We show that GrxS12 is able to catalyze deglutathionylation of different substrates including proteins through a monothiol mechanism requiring only the most N-terminal cysteine of the enzyme. The reaction with glutathionylated substrates proceeds by a ping-pong mechanism. The p$K_a$ of GrxS12 catalytic cysteine is very low (3.9) and makes GrxS12 itself sensitive to oxidation by $H_2O_2$ and to direct glutathionylation by GSSG, GSH plus oxidants and nitrosoglutathione (GSNO). Glutathionylated-GrxS12 (GrxS12-SSG) is temporarily inactive until it is deglutathionylated by GSH in a slow reaction that limits the overall process. Based on the equilibrium between GrxS12 and glutathione ($E_{m(GrxS12\text{-}SSG)}$= -315 mV, pH 7.0), GrxS12-SSG is predicted to accumulate *in vivo* under conditions of mild oxidation of the GSH pool that may occur under stress. Moreover, GrxS12-SSG is predicted to be more stable in chloroplasts in the dark (pH~7.0, $K_{ox}$ 309) than in the light (pH~7.9, $K_{ox}$ 69). These peculiar catalytic and thermodynamic properties could allow GrxS12 to act as a stress-related redox sensor allowing glutathione to play a signaling role through glutathionylation of GrxS12 target proteins.

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**Poster Communication Abstract – 9.39**

# TOMENTELLOID FUNGI: AMONG ONE OF THE MOST ABUNDANT AND DIVERSE ECTOMYCORRHIZAL MYCOBIONTS IN TRUFFLE ORCHARD

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*Tomentella, Pseudotomentella, Tuber aestivum, ectomycorrhizae, ITS sequencing*

Tomentelloid fungi are a resupinate group of mycobionts belonging to the Thelephoraceae (Basidiomycota) and forming ectomycorrhizae with roots of angiosperm and gymnosperm. These fungi are among all the most abundant and diverse taxa in ectomycorrhizal communities from arctic regions to the tropics. Thelephoraceae species belong to *Tomentella* and *Pseudotomentella* genus were recently shown to be co-occurent of the ectomycorrhizal community proper of cultivated and natural truffle environments. Although their morphotypes should be distinguished by typical morphological-anatomical characteristics (colour; surface network; mantle cells organization; emanating elements; rhizomorphs and cystidia) these traits cannot be even used as reliable indicators because they are very susceptible to change under changing environmental conditions.

Here we report the identification of *Tomentella* and *Pseudotomentella* ectomycorrhizae found in a *Tuber aestivum* Vittad. cultivated orchard by PCR-amplifying and sequencing the ITS nrDNA (internal transcribed spacer nuclear ribosomal DNA). Similarities with known sequences were searched in the National Center for Biotechnology Information (NCBI) database using BLASTN application. All ITS sequences were then aligned with 37 additional congeneric ITS sequences retrieved from GenBank and used for phylogenetic and molecular evolutionary analysis using MEGA version 4 software.

In our case study, with 11 different OTUs out of 29 totally detected, Thelephoraceae spp. are the most diverse and predominant mycobionts of the *T. aestivum* truffle orchard. The NJ (neighbour-joining) tree reported revealed the relationships among the identified *Tomentella* and *Pseudotomentella* lineages.

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**Poster Communication Abstract – 9.40**

# GENETIC DIVERSITY OF ALMOND CULTIVARS AND CHARACTERIZATION OF SELF-INCOMPATIBILITY ALLELES

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# These authors contributed equally to this work

*Almond, microsatellites, self-incompatibility*

In Sardinia there is a considerable number of local varieties of almond *(Prunus amygdalus)*. A collection of 43 varieties has been recently characterized both by phenotypic traits and SSR markers. The opportunity to know the genetic diversity of cultivars and different populations may be essential in breeding programs, germplasm management and to optimize the biodiversity valorization.

The Sardinian collection was compared to another collection of genotypes sampled in Southern Italy, mostly from Apulia, and to some international commercial varieties.

Moreover we started to study the self incompatibility (SI) of Sardinian cultivars, in order to enrich their characterization. Cross incompatibility (a well known phenomenon in almond) imposes severe difficulties on commercial production. Until now, 31 SI RNase alleles have been identified in almond.

The 11 SSR markers produced a mean number of 14.5, 11.9, 5.6 alleles per locus for Sardinian, Southern Italy and commercial varieties respectively. Mean expected Heterozygosity (*He,* Nei's, 1973) was 0.86, 0.81 and 0.67 for Sardinian, Southern Italy and commercial varieties respectively. The genetic structure of the populations was studied both by model and distance based approaches. Genetic distance analysis (TREECON) almost coincided with model based analysis (STRUCTURE). Sardinian group, Southern Italy group and commercial cultivars were clearly distinguished, although some exchange occurred: Truoito B and Pititcchedda (Sardinia) resulted closer to the commercial group, whereas Pizzuta d'Avola (Southern Italy) and Picantili (commercial) resulted closer to Sardinian group.

As to self incompatibility, we carried out both the amplification of Sardinian cultivar S-genotypes with allele-specific primers (Tamura M. et al., 2000) and the set up of techniques to amplify the 1st and 2nd intron regions of S-RNase gene, using degenerate primers (Halasz J. et al., 2008). Sequence analysis of amplicons is in progress, and preliminary results are discussed.

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**Poster Communication Abstract – 9.41**

# GENETIC ANALYSIS OF TWO RARE SARDINIAN ENDEMIC OF THE *CENTAUREA* GENUS

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*Centaurea, endemic species, environmental changes, conservation genetics, microsatellites*

Increasingly rapid environmental changes, caused by human activities at the local- and global scale, are expected to impact wild plant populations by altering their fitness to the environment they presently occupy. This is especially true for narrow endemic plant species, because their genetic structure could reveal high variability in a restricted geographic range and the presence of peculiar genotypes not found elsewhere.

In this study, we have considered *Centaurea filiformis* Viviani and *C. ferulacea* Martelli, two rare chasmophytic endemic entities exclusively found in the impervious Mesozoic limestone regions of central-western Sardinia, where they are distributed in a few scattered populations. These populations usually occur on cliff faces included in the wider protected areas belonging to the Natura 2000 European network. In Sardinia the genus *Centaurea* is represented by three other endemic species.

The aim of the present study is the genetic investigation of the remnant population of these species, in order to plan conservation strategies.

To this purpose we sampled 10 populations of *C. filiformis,* representative of the whole habitat of the species, for a total of 180 plants, which were then genotyped at 10 heterologous microsatellite loci. We also sampled and analyzed by the same markers two populations (40 plants) of the congener *C. ferulacea* and two smaller populations composed of individuals that display intermediate phenotypes between the two species. The presence of these phenotypes is not surprising, given that plants with intermediate morphology were also found in a previous study between *C. horrida* and C. *filiformis*, suggesting that interspecific hybridization could have played a significant role in the evolution of several sections of the *Centaurea* genus. In this work genetic differentiation, the presence of a genetic structure and of a spatial genetic one will be investigated.

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**Poster Communication Abstract – 9.42**

# GENETIC DIVERSITY AMONG GRAPEVINE CULTIVARS FROM THE IONIAN COAST OF REGGIO CALABRIA

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*Simple Sequence Repeat, local genotypes, synonymies and homonymies, Vitis vinifera L.*

Grapevine (*Vitis vinifera* L.) is one of the oldest agricultural crops cultivated to produce mainly table fruits and wine. The number of different varieties held in worldwide germplasm collections is estimated to be 10.000 (Alleweldt and Dettweiler, 1994 – The genetic resources of *Vitis*: world list of grapevine collections, 2^ edn. Geilweilerhof, Siebeldingen). This wide genetic diversity is probably due to several mechanisms such as multiple domestication events from wild vines and the old practice of growing seedling from spontaneous or controlled crossing. The vegetative propagation of grapevine frequently produces clones genetically identical to the parental plant, but spontaneous mutations can occur in the regenerative cells that give rise to different clones.

A total of fifty-six grapevine genotypes were sampled from several sites of the Ionian areas of Reggio Calabria Province from Calabria, considered an important casket of biodiversity, where old vineyards are widely spread. The grapevine collection was selected as a representative sample of genetic variability from that area. Total genomic DNA was extracted from young fresh leaves and genetic characterization was performed using twelve of the most utilized microsatellite markers (VVS2, VVS5, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD6 VrZAG62, VrZAG64, VrZAG67 and VrZAG79) for grapevine. The goal of this work is to evaluate the level of biodiversity and to clarify proposed cases of synonymies and homonimies, in order to safeguarding the still existing diversity. Several varieties sampled in the present study had their names quoted in historical and literary sources, while others found and sampled in old vineyards had their names not reported in ancient literature. According to their genetic profiles at SSR loci, 33 different genetic profiles were found, in which several cases of synonymies (Nerello Sculli, Nerello Lamezia, and among different presumed clones of Magliocco Dolce and Malvasia) and cases of homonymy (Olivella and Marcigliana) were discovered. Several genetic parameters, such as expected ($H_e$) and observed ($H_o$) heterozygosities, polymorphic information content (PIC) and probability of identity (PI) were evaluated to assess the efficacy of the chosen loci for the analyses. Pairwise genetic distances between all genotypes analyzed were calculated. A dendrogram representing the genetic similarities among genotypes was obtained using the UPGMA method to investigate their relationships, explaining them from an historical point of view. The genetic analysis confirmed the supposed high level of diversity in this Mediterranean area. The cluster distribution of varieties did not reflect their geographic distribution, suggesting different and successive introductions of cultivars in this area of Calabria region from different areas of origin.

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**Poster Communication Abstract – 9.43**

# TRANSCRIPTOME ANALYSES OF $O_3$ –RESPONSIVE GENES IN LEAVES OF TWO DIFFERENTIALLY SUSCEPTIBLE POPLAR GENOTYPES

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*Acute ozone stress, transcriptome, microarray, poplar clones, ozone-responsive genes*

When subjected to episodic ozone peaks, the more sensitive trees can undergo conspicuous molecular and physiological changes that frequently result in foliar lesion formation. These events involve programmed cell death and other events typical of hypersensitive responses triggered by pathogens or abiotic stressors.

By using the microarray technology, a transcriptome investigation was carried out on the leaves from two poplar clones exhibiting a contrasting susceptibility in terms of leaf injuries after an acute ozone exposure, with the aim to understand the molecular events at the base of foliar lesion formation. Unfumigated plants were used as controls. The microarray platform consisted in a collection of cDNAs extracted from different organisms subjected to abiotic (ozone and cold stress) or biotic (ceratoplatanin phytotoxic protein) stresses.

The genes modulated by ozone were compared with those of the untreated sensitive and tolerant clones. Out of the 337 genes, 119 and 41 genes were evidenced to be specifically $O_3$-responsive in Eridano and in I-214 poplar clones respectively. In both the genotypes (but especially in the Eridano clone), the down-regulated genes were higher in number than the up-regulated ones. Interestingly, sensitive and resistant genotypes evidenced some genes specifically up or down expressed only in one of the two clones. These genes could play a key role in determining the different behaviour displayed by the two clones when exposed to acute ozone stress and constitute an intriguing opportunity to better understand the molecular bases of ozone stress tolerance and sensitivity.

The differentially expressed genes were also compared in sensitive clones with respect to tolerant counterpart at different experimental time points (before ozone exposure, at fumigation end, and during the recovery times). The obtained results evidenced that about the 22% of all transcripts were differentially regulated overtime in the two clones: the majority of these belonged to cell metabolism (primary and secondary) and disease/defence functional categories and resulted mainly down-regulated in the sensitive clone than in the tolerant one. At 5 hrs treatments and during the subsequent recovery periods, the categories having the major number of differentially transcribed genes were those related to cell metabolism and signal transduction pathways. A significant increase in expressed genes from disease/defence and protein synthesis categories was evidenced after ozone stress.

Considering the differences displayed by the two clones in the expressed transcriptome, we suggest that a more or less efficient deployment of defence mechanisms minimizing the toxic effect of $O_3$ or its by-products can explain the differences in ozone sensitivity showed by the two poplar hybrids. The transcription profiles, obtained by using these cDNA microarray platforms, indicated that several genes differentially regulated by ozone in Eridano and I-214 poplar clones were the

same regulated by other abiotic or biotic stressors in different organisms, underlying the existence of a conserved and interspecific network of genes, activated during plant defence responses.

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**Poster Communication Abstract – 9.44**

# NEW RESULTS ON THE GENETIC DIFFERENTIATION BETWEEN POPULATIONS OF SCOTS PINE (*PINUS SYLVESTRIS* L.) FROM SEVERAL GEOGRAPHIC REGIONS OF ITS NATURAL RANGE

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*Population genetics, Pinus sylvestris, genetic diversity, genetic differentiation, natural range*

Scots pine (*Pinus sylvestris* L.) occupies a larger natural range than any other species from the whole *Pinaceae* family, extending from Europe to the Far East (Manchuria) through Siberia. Because of such a wide geographic spreading, with very different environmental conditions, and because of the long evolutionary history of this pine, a large intraspecific variation is expected to occur. The aim of this research is to study the genetic diversity and the differentiation between populations representative of the Italian and of the remaining Eurasian natural range of Scots pine; in the case in point, eight Italian populations, 22 populations from the rest of Europe (nine of which are French) and one from Asia (Turkey), for a total of 31 populations, were studied by using isozymes as genetic markers, analysed through horizontal starch gel electrophoresis. The obtained results confirm the previously observed sharp differentiation of an Italian population, located in the Emilian Apennine: it is a relict and isolated remnant from glacial migrations, and it is even less similar to the studied Italian Alpine populations than the remaining foreign populations, which tend to group together with them. These new observations supply further evidence of the status of important genetic resource for this small and autochthonous stand, whose differentiation could depend both on its origin from a different glacial refugium and on a different evolutionary history, and whose values of genetic diversity parameters are similar to those found in the other Italian populations, in spite of its geographic isolation from the main range of this species. On the basis of the obtained values of genetic distance, the seven Italian populations from the Alps tend to group together and appear rather differentiated from the remaining ones, suggesting both a different postglacial origin and a relative genetic isolation due to the Alpine barrier, but their cluster is close to a group of French populations, suggesting a common origin and a subsequent differentiation. Other French populations are scattered along the dendrogram. Some hypotheses on the postglacial recolonization routes followed by this species are also discussed. The results of this research are increasing the available knowledge on Scots pine, making it possible the drafting of more accurate programmes of genetic resource conservation.

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**Poster Communication Abstract – 9.45**

# ORIGINS AND EVOLUTION OF THE ETRUSCANS' DNA

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*Ancient DNA, mitochondrial DNA, Approximate Bayesian Computation*

The Etruscan culture is documented in Etruria, Central Italy from the 7th to the 1st century BC. For more than 2,000 years there has been disagreement on the Etruscans' biological origins, whether local or in Anatolia. Genetic affinities with both Tuscan and Anatolian populations have been reported, but so far all attempts to fit the Etruscans' and modern mitochondrial DNAs in the same genealogy have failed. In this study we expanded the ancient sample, typing 14 individuals from the Tarquinia and Casanievole necropoleis, according to all the standard criteria to ensure reproducibility of the ancient DNA sequences. We analysed these sequences along with previously typed Etruscan and Tuscan Medieval sequences from our lab, and with a wide database of modern European populations.

We compared ancient and modern mtDNA diversity with the results of millions of computer simulations by methods of Approximate Bayesian Computation. In this way, we identified the demographic model showing the closest agreement with the observed data, and we estimated its relevant parameters. We found significant evidence of genealogical continuity between the Etruscans and two communities from Tuscany, Volterra and Casentino, whereas the results about a third Tuscan isolate, Murlo, were more ambiguous. People of coastal Anatolia, the area where ancient historians placed the Etruscans' putative roots, appear descended from ancestors who had some degree of genetic similarity with the Etruscans. However, estimates based on a model of isolation with migration suggest that these similarities date back to <10,000 years ago, and hence originated long before the appearance of the Etruscan culture in the archaeological record. If confirmed, this result would strongly suggest that the Etruscan culture developed locally without significant contribution of recent Anatolian immigrants.

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**Poster Communication Abstract – 9.46**

# CYTOGENETIC SURVEY IN SOME ENDANGERED ANIMAL SPECIES REARED IN CAMPANIA REGION

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*Cattle, pig, endangered breed, chromosome abnormality, biodiversity*

The use of cosmopolitan animal breeds has been radically changing the genetic patrimony accumulated in thousands of years in some local breeds as a result of full integration between genotypes and environments. Indeed, in Italy (and Campania region) the selection of breeds with high genealogy and productions has been producing a continue reduction of local breed animals which most often are correlated to local and typical products.

In the present study a cytogenetic survey has been performed on 60 cattle (*Bos taurus*, 2n=60) from endangered breeds (of which 20 from Agerolese breed) and 15 pig (*Sus scrofa*, 2n=38) from Casertana breed raised in Southern-Italy at the ConSDABI center, to check for the presence of chromosome abnormalities.

Peripheral blood cell cultures were performed without (normal cultures) and with addition of 5-Bromodeoxyuridine (BrdU) during the last 6 h of cell culture to obtain R-banding chromosome preparations. Slides obtained from normal and BrdU-treated cells were used for C- and R-banding techniques, respectively. For some animal, fluorescence in situ hybridization (FISH) technique and bovine BAC-clones, as probes, were employed.

While no chromosome abnormalities were found in pig, the following chromosome abnormalities were found in five cattle (8.3 % of investigated cattle): (**a**) XX/XY chimera (freemartin) in two females from Agerolese and Modicana breeds which resulted both sterile for internal gonadal dysgenesis; (**b**) rob(1;29) at the homozygous (2n=58) and heterozygous (2n=59) conditions in two females of Garfagnina and Varzese-Ottonese breeds, respectively; (**c**) a new and unusual reciprocal translocation in a female cattle of Agerolese breed involving chromosomes 11 and 25, as demonstrated by both CBA- and RBA-banding techniques, as well as by FISH-mapping using specific molecular markers of cattle chromosomes 11 and 25.

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**Poster Communication Abstract – 9.47**

# *UCP3* POLYMORPHISMS, HAND GRIP PERFORMANCE AND SURVIVAL AT OLD AGE

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*Uncoupling proteins, hand grip, longevity*

An efficient uncoupling process is generally considered to have a protective effect on the aging muscle by slowing down its age-related decay. Genetic polymorphisms in the Uncoupling Protein 3 (*UCP3)* gene, whose product is mainly expressed in skeletal muscle, were suggested to be associated with hand grip performances in elderly populations. In our work, we aimed to add further support to this evidence by analyzing the correlation between four SNPs in the *UCP3* gene and relative haplotypes, in two large cohorts of middle aged and oldest old Danes (N=1616). We found that the variability at two SNPs significantly influenced hand grip performance in both cohorts. Consistently, SNP combinations including significant alleles in single-locus analysis resulted in different haplotypic associations with hand grip performance. Finally, taking advantage of large cohort and period survival data of the oldest cohort, we tested the association of each SNP with survival at 10 years from the baseline visit. Interestingly, we found that alleles associated with hand grip scores showed differential survival patterns, with people carrying the allele negatively influencing hand grip phenotype showing also higher mortality in our oldest cohort. On the whole, our work supports the role of *UCP3* gene in functional status and survival at old age.

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**Poster Communication Abstract – 9.48**

# THE GENE VARIABILITY OF NEURONAL NITRIC OXIDE SYNTHASE AFFECTS COGNITIVE FUNCTIONING AND SURVIVAL IN THE ELDERLY

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*Aging, cognitive decline, nitric oxide, NOS-1*

Nitric oxide (NO) is an important endogenous mediator involved in the regulation of the cardiovascular, nervous and immune systems. In mammals, NO is synthesized by a complex family of enzymes named NO synthases (NOS), encoded by 3 distinct NOS genes, including: neuronal (*nNOS or NOS-1*), inducible (*iNOS or NOS-2*) and endothelial (*eNOS or NOS-3*). The regulation of NO production is a crucial process to ensure homeostasis in tissues and apparatus. NO is involved in the regulation of many metabolic pathways, and in particular seems to play a crucial role in the process of brain aging. Indeed, at the level of CNS, NO, synthesized by *NOS-1*, acts as neurotransmitter, neuromodulator, or intracellular signaling molecule. The variability of *NOS-1* gene has been proved to affect both pathological and normal phenotypes correlated to cognitive function. Since the maintenance of cognitive abilities is an important factor for successful aging and it is a major component of the quality of life in the elderly, we tested the association between two selected SNPs (rs1879517 and rs2683826) falling in the *NOS-1* gene and the preservation of cognitive function in the elderly, and its possible effects on survival. Cognitive function was measured by Mini Mental State Examination (MMSE), corrected for age and school-attendance rate. A sample of 624 subjects from southern Italy (age range 60-107 years) was screened for *NOS-1* variability. We found that within the 65-89 years age range (the prevalence of cognitive impairment is greatest in this age group) the C/C genotype relative to the rs1879517 is overrepresented in subjects with impaired cognitive function (MMSE < or = 23) compared to those with conserved cognitive function (MMSE > 23) ($p=0.04$). As cognitive functions have a crucial role in survival chance in the elderly, the correlation between these polymorphisms and survival was then analyzed in a larger sample divided into two specific age groups (subjects aged from 60 to 85 years and subjects aged over 85). A significant association was found under the recessive model for minor allele C of rs1879517 ($p=0.004$) suggesting the C/C genotype to be detrimental for survival in the elderly. The rs1879517 variant it is located in the promoter region, but there are no data indicating its functional role. *In vitro* studies will be carried out to clarify this point.

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**Poster Communication Abstract – 9.49**

# A MULTILOCUS ANALYSIS SHOWS THE ROLE OF GENETIC VARIABILITY OF GENES INVOLVED IN METABOLIC PATHWAYS IN HUMAN LONGEVITY

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*Longevity, metabolic pathways, multilocus longevity*

Many studies on both animal models and humans have shown that metabolic pathways (INS/IGF-1; GH; cell cycle; metabolism of xenobiotics etc.), conserved along evolution, play a crucial role in lifespan extension. In order to highlight if, and to what extent, the variability of the genes involved in metabolic pathways affects human longevity we examined the variability of numerous genes involved in these pathways in a population from Calabria.

The study was a two step multilocus association analysis to detect genetic variants that affect human longevity. The sample analyzed was composed of 991 (514 female and 476 males; age: 50-104), unrelated subjects from Calabria. About 15% of the samples (149 subjects: 69 cases and 64 controls) were analyzed in Stage 1 for 305 SNPs (Single Nucleotide Polymorphisms) belonging to 105 genes selected from different metabolic pathways (INS/IGF-1; GH; cell cycle; metabolisms of xenobiotics; stress response and Neuro active ligand-receptor interaction). In Stage 2 we have genotyped the remaining samples (240 cases and 529 controls) for 27 [0]SNPs selected from those belonging to Stage 1. Both dataset were analyzed for association with longevity by using MAX3 test.

We found that two polymorphisms were associated with longevity. One is located in a gene that codifies for a protein member of the serine/threonine protein kinase family. This kinase mediates the signaling transduction and controls a variety of cell functions including transcription and apoptosis. A second polymorphism associated [0]with longevity falls in a gene that is a member of the glutathione S-transferase (GSTs) super-family. These proteins have a crucial role in metabolisms of xenobiotic and in particular in the detoxification of carcinogens, mutagens. Moreover, it is also involved in the response to oxidative stress. This polymorphism is located in the intronic region (introne 1), very close to the 5'UTR region. So it is possible that this polymorphism falls in a regulatory region (for example a transcription factor binding region) or that is in LD with another functional SNP. HapMap data shows that this SNP is in a strong linkage with other polymorphisms located in intronic region or in 5'UTR region.

Future studies are needed to better explain the role of this polymorphism in longevity.

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**Poster Communication Abstract – 9.50**

# THE ROLE OF SPHINGOLIPID METABOLISM GENES IN THE RESPONSE OF *SACCHAROMYCES CEREVISIAE* CELLS TO STARVATION FOR ESSENTIAL NUTRIENTS

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*Saccharomyces cerevisiae, nutritional stress response, sphingolipid metabolism genes, autophagy*

The goal of the presented research has been to investigate the possible role of genes involved in the sphingolipid metabolism in the response of the yeast *Saccharomyces cerevisiae* cells to environmental stresses such as starvation for essential nutrients (aminoacids or nitrogen bases for auxotrophic strains). We focused on the genes ISC1, SUR1, SUR4, IPT1, CGS2 and SCS7 whose products are known to be fundamental for a proper response of yeast cells to various stressful conditions. First we compared the survival of the auxotrophic yeast strain BY4741 (Mat a *his 3Δ1 leu 2Δ0 ura 3Δ0 met15Δ0*) starved for leucine with that of isogenic strains deleted in the above mentioned genes. The survival was determined by spot tests and survival curves at different times (1-2-3 days) of starvation. Our results showed that the strain defective in the gene ISC1( *ISC1Δ*) was much more sensitive than the others to leucine starvation. Then we tested the strain *ISC1Δ* for histidine, methione and uracil starvation confirming its higher sensitivity with respect to the wild-type strain. On the basis of these results we conclude that the product of the gene ISC1 is involved in the response of yeast cells to nutritional stress. To understand the role of ISC1 during starvation for nutrients we wondered if its deletion could jeopardize the possibility of yeast strain to adopt the proteolytic pathway of autophagy which is known to extend yeast cells chronological longevity when they are starved for aminoacids. To monitoring the autophagy process we applied a method based on the unique properties of the fluorescent dye FM 4-64 to follow the accumulation of autophagic bodies. In a preliminary experiment autophagy was monitored in wild-type and *ISC1Δ* cells grown for 4 hours in minimal medium without any essential aminoacids and without uracil. In this condition we found that *ISC1Δ* cells cannot induce autophagy compared to BY4741 wild type strain. The next steps will be to monitor autophagy in wild-type and *ISC1Δ* cells under the same experimental conditions used for the determination of survival. This could help to find a possible correlation between the autophagy-deficient phenotype of *ISC1Δ* cells and their higher sensitivity to the essential nutrients deprivations tested.

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**Poster Communication Abstract – 9.51**

# MITOCHONDRIAL DNA HAPLOGROUP R IN MODERN CATTLE: A CONTRIBUTION OF ITALIAN AUROCHSEN?

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*Bovine mitochondrial DNA, domestication, European aurochsen, Bos primigenius*

The debate on the genetic contribution of European aurochsen to taurine cattle gene pool after the generally agreed Neolithic domestication that occurred in the Fertile Crescent from local *Bos primigenius*, is still open. We have sequenced the D-loop of 2032 taurine cattle from 40 European, 3 Egyptian and 7 Ethiopian breeds confirming the overall clustering within haplogroups of Near Eastern ancestry (T1, T2, T3 and T5), but also identifying 28 mtDNAs (1.4%) not clustering within haplogroup T. Complete mtDNA sequencing of non-T samples revealed 10 subjects belonging to the novel haplogroup R, which represents a very early split (~135 ky) in the mtDNA phylogeny of *B. primigenius*. The remaining 18 samples clustered within the recently discovered haplogroup Q. Phylogeographic data indicate that R mtDNAs might derive from female aurochsen of the Italian Peninsula sporadically included in domestic herds, whereas Q and T subclades were most likely involved in the same event of Neolithic domestication in the Near East.

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**Poster Communication Abstract – 9.52**

# STRUCTURAL VARIABILITY IN GENE PROMOTER

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*Gene promoters, phylogensis, Shannon entropy, base composition*

In a previous paper (Calistri et al., 2011), we analysed GC/AT ratios along the 1000 nucleotide sequences upstream of the TSS in wide sets of promoters belonging to organisms ranging from bacteria to pluricellular eukaryotes and showed very clear phylogenetic trends throughout evolution of promoter sequence base distributions. We are now interested to search a putative correlation between the increase in organismal complexity observed during phylogenesis and the increase of variability in their promoter structures. To test this hypothesis we have introduced a suitable spatial entropy indicator (Positional Shannon Entropy), which allow the measure of the variability content in promoters sets. Every species examined shows its own specific entropy along the sequences and shows regions with different levels of information. By selecting our sequences according to the presence/absence of the TATA-box or comparing housekeeping versus tissue specific or single versus alternative promoters, we obtain, in higher vertebrates, structurally different classes of sequences.

Thus, promoter functional differences correlate with structural differences, which can also be appreciated through the analysis of the base distributions along promoters, by which we manage to identify different structural categories, the composition profiles allowing a division between genes with a small density gradient and others with a straight one.

This would be in accordance with the progressively more elaborate regulation of gene expression systems that seem to account for organism's complexity and the widening of the functional variability spectrum. We show an increase in intraspecific structural diversification among promoters during evolution: more complex organisms, that is the ones with a higher number of differentiated tissues or stages of development, possess more diversified promoters. We hypothesize that heterogeneity in structure may correspond to functional variability, which has an adaptive value, as variable gene expression helps to cope with variable environments and/or changes during development and might also favour adaptation during evolution.

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**Poster Communication Abstract – 9.53**

# PROTEIN PHOSPHATASE 2A (PP2A) IS REQUIRED FOR THE MAINTENANCE OF *DROSOPHILA* CHROMOSOME INTEGRITY

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*PP2A, chromosome aberrations, DNA repair foci, cell cycle*

The processes through which cells sense and repair DNA lesions are collectively known as DNA damage response (DDR). Although Ser/Thr protein kinases have pivotal roles in the DDR, growing evidence indicates that these kinases and their substrates work in concert with numerous Ser/Thr phosphatases. One of the DDR key events is phosphorylation of the histone variant H2AX (H2Av in *Drosophila*) at the sites of DNA breakage to a form γ-*H2AX*, which recruits several additional DNA repair factors. These factors form discrete nuclear foci that dissolve when DNA repair is completed. Recent work has shown that completion of DNA repair requires dephosphorylation of γ-H2AX and that several phosphatases participate in this event. However, a direct evidence for a role of phosphatases in the maintenance of chromosome integrity is still lacking. We have isolated a lethal mutation, $tws^{430}$, in the *Drosophila twins (tws)* gene, that encodes the B regulative subunit of the Ser/Thr phosphatase 2A (PP2A). This mutation causes frequent (54%) chromosome aberrations (CAs) in larval neuroblasts. In addition, $tws^{430}$ mutations affect the regression of IR-induced repair foci; in $tws^{430}$ mutant brains the γ-H2Av foci persist much longer than in controls, suggesting that PP2A is required for γH2Av dephosphorylation. In $tws^{430}$ mutants, the cell cycle does not slow down after IR-induced DNA damage. The mitotic index (MI) of wild type brains showed a strong decrease 15' after irradiation and remained lower than that of non-irradiated controls for two hours. In contrast, in irradiated $tws^{430}$ mutant brains the MI was consistently similar to that of non-irradiated controls. These data indicate that PP2A may have a role also in the G2/M checkpoint. Double mutant analysis showed that mutations in *tefu* (ATM) are epistatic over mutations in *tws* (PP2A); in contrast *mei-41* (ATR) *tws* double mutants showed a significantly higher frequency of CAs than either single mutant. One appealing interpretation of these results is that *Drosophila* PP2A is primarily involved in dephosphorylation of ATM substrates, and that lack of *tws* activity results in the presence phosphorylated proteins that interfere with the normal DNA repair processes.

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**Poster Communication Abstract – 9.54**

# THE CITRATE LYASE REGULATES CELL DIVISION DURING *DROSOPHILA* MALE MEIOSIS

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*Drosophila*, *male meiosis, cytokinesis, chromosome, ACL*

The Citrate Lyase (ACL) is the main cytosolic enzyme that converts the citrate exported from mitochondria, in acetylCoA and ossalacetate. As Acetyl-CoA is an essential substrate for the biosynthesis of cholesterol and long-chain fatty acids, ACL acts as critical enzyme for the de novo synthesis of a wide range of complex cellular lipid. In addition, ACL provides acetyl-CoA for histone acetyltransferase in the cell nuclei. We have isolated a P-element induced male sterile mutation in the Drosophila *DmACL* gene that encodes the ortholog of human ACL, suggesting a role of ACL during male meiosis and spermatogenesis in fruit flies. *in vivo* analysis of mutant testes revealed the presence of multinucleate spermatids and micronuclei. More than 80% (n= 120) of mutant spermatids exhibited an irregular association between the nebenkern, a mitochondrial derivative, and nuclei indicating that loss of ACL gives rise to a failure of cytokinesis in male meiosis. In addition, 20% of spermatids exhibited also nuclei of different sizes, suggesting that chromosome segregation is also affected upon depletion of ACL. Immunolocalization of a-tubulin and the centriole component Spd2 revealed the presence of multipolar spindles during both meiotic divisions. Particularly, we found an high proportion of anaphase I cells (80%; N=50) with either bipolar, tripolar ("bugs bunny-like" configurations) or quadripolar spindles that contained four distinct nuclei each denoting a cytokinesis defect occurring during primary spermatocyte formation. DAPI staining of mutant meiotic chromosomes showed also the presence of either lagging chromosomes and chromatin bridges which are likely to give rise to micronuclei in spermatids. Here we present data suggesting that depletion of ACL causes 1) cytokinesis failure because of alterate membrane synthesis in male meiotic cell division and 2) chromosome segregation defects as consequence of an umbalanced acetylation status of chromatin.

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**Poster Communication Abstract – 9.55**

# THE CITRATE LYASE PLAYS CONSERVED ROLES IN THE MAINTENANCE OF GENOME STABILITY

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*Drosophila, ACL, chromosome segregation, genome stability, histone acetylation*

We have previously shown that *scheggia (sea),* which encodes the fly ortholog of mammal mitochondrial citrate carrier SLC25A1, is required for preventing chromosome breaks in *Drosophila* and humans. To further investigate the conserved cross-talk between citrate metabolism and maintenance of genome stability, we have started to genetically dissect the pathway that yields cytosolic Acetyl-CoA formation from citrate. We thus focused on the ATP citrate lyase (ACL) enzyme that converts the citrate exported from mitochondria, in acetylCoA and ossalacetate. We found that in homozygous and hemizygous ACL mutant combinations histone acetylation was reduced. Moreover, mutant larval brains exhibited a significant proportion of hyperploid and tetraploid cells as well as chromatid and isochromatid breaks. In addition, a small portion of mutant anaphases were abnormal with respect to wild-type in that they displayed chromatin bridges and lagging chromosomes which are likely acentric. However, in contrast with extensive chromosome breakage elicited by *sea* mutants, depletion of DmACL gave rise to rare, spontaneous chromosome breaks (3% *DmACL,* N= 180), suggesting that loss of either Sea or ACL affects genome stability in different ways. Interestingly, feeding *DmACL* mutant larvae with 0.5 M citrate partially abolished chromosome defects indicating that, as observed previoulsy for *sea* mutants, intracellular levels of citrate can regulate chromosome dynamics in mitosis. To check if the mitotic defects influenced mitosis progression, both frequency of anaphases (AF) and mitotic index (MI) have been determinated. We have observed that *DmACL* mutants do not display any changes in either AF or MI mitotic parameter suggesting that chromosome segregation defects and breaks do not activate either SAC or DDR, respectively. Altogether, these observations indicated that DmACL function is required for a proper completion of mitosis in *Drosophila* mitotic cells.

Owing the high degree of conservation between DmACL and human ACL, we sought to determine whether ACL depletion affected chromosome behavior also in human primary fibroblasts. Very interestingly, human fibroblasts transfected with siRNA duplexes against ACL exhibited a significant number of hyperploid/tetraploid cells (30%; N= 100). As this phenotype is very similar to that elicited by *DmACL* mutant cells, we can conclude that DmACL/ACL, plays an evolutionary conserved role in controlling chromosome stability.

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**Poster Communication Abstract – 9.56**

# UNRAVELING GENETIC TRACES OF ANCIENT MIGRATORY MIDDLE EASTERN ROUTES: A SURVEY OF Y-CHROMOSOME VARIATION IN IRAN

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*Y-chromosome, Iranian population, SNPs, STRs*

Iran is one of the main southwestern Asian countries and had a central role in human evolution. Its geographic location between the Caspian Sea in the north and the Persian Gulf in the south made it an important passageway for the early spread of modern humans from Africa to Asia. It was also one of the regions in which agriculture had origin about 10,000 years ago, home of the first urban civilizations, subject to the arrival of Indo-Iranians in the 2$^{nd}$ millennium and, in historical time, home of big empires and invaded by several populations such as Arabs, Mongols and Ottoman Turks. At present, the Iranian population is an interesting mix of more than 100 different ethnic groups speaking a variety of Indo-Iranian, Semitic and Turkic languages. Arabs, Armenians, Assyrians, Azeris, Baluchs, Bandaris, Gheshmi people, Gilaks, Kurds, Lurs, Mazandarani, Persians, Turkmens, Zoroastrians and a group of so called Afro-Iranians, who might be the result of the slave trade with Zanzibar, are the most represented ones.

Despite this scenario is of great interest to reconstruct traces of ancient migrations, only few studies (based on small sample sizes and a low resolution genotyping) have investigated the gene pool of modern Iranians.

To shed some light on the genetic structure of this Levantine population and on the ancient migrations that affected this area, 930 Iranian male DNAs belonging to 15 ethnic groups from 14 Iranian provinces were analysed for Y-chromosome variation.

Due to its uniparental transmission, absence of recombination and wide dataset availability, the Y-chromosome is (together with mtDNA) among the best genetic systems for detecting signs of ancient migrations and for evaluating socio-cultural behaviours.

All the 930 chromosomes belonged to 15 main haplogroups (B, C, D, E, G, H, I, J, L, N, O, P, Q, R and T) the most frequent of which are J (31%), R (29%), G (12%) and E (9%) with great differences in frequencies and sub-haplogroup distribution among provinces and ethnic groups. The comparison of the Iranian haplogroup frequencies with those of neighboring Asian, European and African populations allowed to identify some external contributions (for example: the sub-Sahara African Hg E-M2 in Southern Iran and the Central Asian Hgs H and Q in Eastern Iran) and to evaluate the extent of the *in situ* differentiation of the autochthonous haplogroup J.

Overall, the results of this study provide an accurate and reliable portrait of the Y-chromosomal variation in the Iranian region, useful for generating a more comprehensive history of the peoples of this area as well as for reconstructing ancient migration routes.

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**Poster Communication Abstract – 9.57**

# EARLY SEX DETERMINATION STEPS IN THE MEDITERRANEAN FRUITFLY *CERATITIS CAPITATA* AND ITS MANIPULATION FOR PRODUCTION OF A MALE-ONLY PROGENY

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*Sex determination, biotechnology, early embryonic development*

In the agricultural pest insect *Ceratitis capitata*, the Mediterranean fruitfly, (Medfly) the sex determination is controlled by a Y-linked male determining factor which influences, either directly or indirectly, the state of activity of the female determining master gene *Cctra*$^{ep}$ (*Cctra epigenetic*) at 5-7 hours from oviposition. We have developed a *Ceratitis* transgenic sexing strain able to produce male-only progeny (95% efficiency) by transgene-mediated RNAi against the female determiner *Cctra*$^{ep}$ gene. *Ceratitis capitata* XX pseudo-males are apparently fully fertile and hence selected XX male flies can be crossed with XX females leading to female only XX progeny. We have prepared polyA+ RNA from XX embryos and from mixed XX/XY embryos, both collected at 5.7 hours from oviposition. We have used a molecular subtractive approach to identify differential expressed genes in XY versus XX at embryonic stages. We will present the molecular strategy employed to approach this problem and preliminary data describing the identification of 8 male-biased cDNA positive clones presently under analysis. Embryonic RNAi experiments are underway to investigate the function exerted by these genes during early embryogenesis of *Ceratitis*.