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Poster Abstract – 7.01

A QUANTITATIVE REAL-TIME APPROACH FOR TESTING TRAIT PURITY IN TRANSGENIC VARIETIES

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trait purity, GMOs, real-time PCR, Roundup Ready soybean

Real-time PCR is widely utilized in the food/feed as well as in the seed sector for the detection and quantification of adventitious presence of genetically modified (GM) contaminants in conventional material. Trait purity is a key factor for the successful utilization of biotech varieties and is currently assessed by analysis of individual seeds or plants. Here we describe a novel approach to test trait purity on bulk samples based on real-time PCR that relies on the detection and quantification of the wild-type (wt) allele sequence corresponding to the insertion site of the transgenic construct. As a proof of concept, we developed a real-time quantitative PCR method to test purity of glyphosate tolerant (Roundup Ready®, RR) soybean. The assay successfully amplified the target wt sequence in all the conventional varieties tested, indicating its ability to detect possibly any soybean non-trait contaminant. The potential occurrence of SNPs and short insertions/deletions within the wt allele was further investigated with a High Resolution Melting (HRM) approach. On the other hand, no amplification product was obtained from any of the RR soybean varieties tested, indicating that the wt sequence is single copy and represents a suitable marker of conventional soybean presence. In addition, results obtained from the analysis of wt-spiked RR samples demonstrate that it is possible to use the real-time PCR assay to quantify the non-trait contamination with an acceptable degree of accuracy.

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Poster Abstract – 7.02

IDENTIFICATION OF PLANT RAW MATERIALS IN FEEDSTUFF BY QUALITATIVE PCR

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traceability, feed, molecular markers, STS, PCR

A simple and not expensive PCR-based method was developed to identify the species included in plant raw material used as feed mixture. Four species-specific genes have been selected to develop as many STS (Sequence tagged sites) markers to identify four target species: Lectin A gene (faba bean), Convicilin A gene (field pea), UDP-glucosyltranferase gene (grain sorghum) and Hordoindoline-a gene (barley). Identification of durum and common wheat (lipid transfer protein gene), soybean (Gly m Bd 30K allergen gene) and maize (invertase gene) was carried out using markers available from the literature. A large number of faba bean (19), field pea (27), barley (24), durum wheat (30), common wheat (25), grain sorghum (26), soybean (24) and maize (2) varieties were analyzed to improve accuracy. Cross-reactivity of the primer pairs was also tested against species that are not usually included in feed mixture, as oat, rye, kidney bean and lentil.

This method was applied for the analysis of flour mixtures. Eight flour samples, one for each species, were used to prepare eight serial dilution of flour mixtures. Within each series, each target species flour was included at different concentrations (0, 0.9, 5, 10 and 100%) while non target species were included at equal amount. The presence of the species included was confirmed by the STS markers as a clear amplification product. The seven STS markers amplified in all mixtures but 0%, where target species was absent.

The efficiency of the method was also verified with an extruded sample mix of soybean, faba bean and field pea where technological treatments could damage DNA integrity. DNA analysis confirmed the presence of the three species included and evidenced a slight maize contamination.

This research, funded by the Marche Region (LR37/99), could be a strategy applicable to traceability and certification of animal feeding systems based on locally produced forages and feeds, within high quality animal production chains, closely linked to production area.

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Poster Abstract – 7.03

DNA MICROARRAY FOR DETECTION OF MAYOR FOOD CONTAMINANTS

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detection, microarray, GM, CustomArrayTM semiconductor technology

Identification and prioritization of effective food safety interventions require an understanding of the relationship between food and pathogen from farm to consumption. Concerns about food safety have played a key role in the emergence of the public health system in the world. Potential hazards may be physical, artificial or naturally-occurring chemicals, organisms which cannot reproduce outside a specified life-cycle (e.g., parasites such as tapeworm in pigs) or viruses. Other microbes reproduce on the surface of food and in the environment. The potential of oligonucleotide microarrays for medical, food safety and biodefense analysis of microbial pathogens is fast unfolding. Microarray has the potential to become a leading trend in bacterial identification in food and feed improvement.

Moreover, the acceptance of GMOs by consumers is still controversial and has pushed the authorities of different countries to implement GMO labelling regulations. The development and application of a reliable and specific simultaneous analytical detection method is thus essential in order to guarantee the consumer's access to information as well as to enforce food labelling by the competent authorities.

For this reason we have developped a new DNA microarray consisting of 4 sub-arrays each containing about 2,000 oligo-probes for the detection of bacteria, fungi, allergens and GM DNA targets in plant. The oligo-probes have been designed and synthesized via *in situ* on chips based on CustomArrayTM semiconductor technology (CombiMatrix Corporation, www.combimatrix.com).

In order to detect and identify more contaminants simultaneously we have also developed an assay for simultaneous detection of bacteria, fungi, allergens and GM DNA using specific PCR primers to amplify variable regions of target genes. The method combines the amplification of several target sequences using various specific primer pairs, followed by their discrimination by hybridization on specific capture probes present on the DNA microarray. Validation tests are currently in progress.

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Poster Abstract – 7.04

COMPARATIVE GENOME ANALYSIS OF APOSTART AND OTHER GENES INVOLVED IN THE CONTROL OF PLANT REPRODUCTION

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APOSTART, apomixis, meiosis, genome organization

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

For understanding the molecular genetics of complex traits such as apomixis, the isolation of specific genes is crucial. With this respect, the use of new cloning strategies could provide innovative tools to isolate genes involved in traits of interest. The choice of the right method is related to the kind of information desired.

We have demonstrated that a cDNA-AFLP strategy, applied to developmental staged inflorescences, was useful to identify several ESTs differentially expressed between apomictic and sexual genotypes of *P. pratensis*. In particular APOSTART, SERK and PpMET showed to be putatively involved in the formation of unreduced embryo sacs and in the modification of sexual reproduction.

We performed a comparative analysis based on bioinformatic approaches to investigate on the genome organization of these genes in *A. thaliana* and in other plant species for which genome sequences are today available.

Our goal was to consider genome distribution of this set of genes, possible functional implications and evolutionary relationships.

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Poster Abstract – 7.05

EXPRESSION OF A MUTATED FORM OF GAD65 IN HETEROLOGOUS SYSTEMS

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T1DM, autoantigen, heterologous systems, enzymatic assay

Type 1 insulin-dependent diabetes mellitus (T1DM) which afflicts 0.2-0.3% of population is caused by autoimmune destruction of insulin-secreting beta cells. The young age of affected patients, the need for life-long insulin therapy and the high prevalence of late-onset complications make T1DM a major health problem. The smaller isoform of glutamic acid decarboxylase of 65 kDa (GAD65) is the major autoantigen in human T1DM and it has recently demonstarated that two injections of the molecule can give protection against this autoimmune disease.

T1DM requires a primary prevention because the disease has a complex genetic basis, making difficult to identify in the population people at risk of developing it. Vaccination studies and subsequent vaccination treatment of a lot of people need large quantity of purified protein, but the current production systems are too much expensive and unable to provide enough GAD65 to meet global demand.

We have previously shown that GAD65 can be expressed in transgenic tobacco plants but yields are disappointing. In order to improve its expression level we use different heterologous systems such as *Nicotiana tabacum* plants, *E.coli* inducible system and insect cells/Baculovirus to express two different forms of the recombinant human GAD65: the wild type form of the enzyme (hGAD65) and the mutated form with no catalytic activity (hGAD65mut), hypothesising that the enzymatic activity might interfere with its accumulation in heterologous systems.

In previous studies it has been demonstrated in *vitro* the lack of the enzymatic activity for the hGAD65mut and we show that GAD65mut accumulates to higher levels in transgenic plants and in *E.coli* inducible system than its enzymatically active counterpart, indicating that the catalytic properties of GAD65 contribute to its poor yields.

To demonstrated the absence of enzymatic activity of the mutated form of GAD65 (GAD65mut) also in the heterologous systems we perform an enzymatic assay in *vivo*,. The results of the assay and the difference among the expression levels obtained in the heterologous systems are discussed.

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Poster Abstract – 7.06

NUCLEAR AND PLASTIDIAL EXPRESSION OF THE GENE ENCONDING HUMAN APOLIPOPROTEIN APOA-I IN TOBACCO

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apolipoprotein, nuclear transformation, plastid transformation, Nicotiana tabacum, pharmaceuticals

Apolipoprotein A-I (ApoA-I) represents 70% of the human plasma high-density lipoproteins, which play a major role in transporting cholesterol from peripheral cells to the liver where it is degraded. Therefore, ApoA-I has been found to play a pivotal role in preventing atherosclerosis development. For therapeutic purposes, we suggest plants or plant cells cultures as an alternative over other transgenic protein production platforms. In a previous SIGA presentation (2004; www.siga.unina.it/SIGA2004/E_14.pdf), we reported the production of several nuclear transgenic tobacco lines after co-cultivation with *Agrobacterium tumefaciens* stably expressing human ApoA-I. In this presentation, we report further characterisation of T1 plants, which were found to express higher amount of ApoA-I than the parental lines. Moreover, the recombinant protein was detected in plant organs at various stages of development, including senescent leaves. Electronic and fluorescence microscopy analyses demonstrated that recombinant ApoA-I was present in the cytoplasm, vacuoles and apoplast of leaf cells. Furthermore, a cell culture was established from a T1 transgenic plant. Recombinant ApoA-I was isolated both from transgenic leaf extracts and from the conditioned culture medium of transgenic cells. An antibody-based one-step procedure was set up to maximise the recovery of transgenic protein from crude or culture medium extracts.

To increase the amount of ApoA-I synthesised in the plant cell and to exploit other potential advantages of the plastid transformation approach, the *apoA-I* gene was also integrated into the plastid genome. Two vectors with different 5'-untranslated regions (5'-UTRs) fused to the promoter (P*rrn*) of the tobacco plastid ribosomal RNA operon were produced. The pPL80 vector contained the phage T7 gene 10 leader sequence, whereas pLS1 the *rbcL* 5'-UTR with an additional 42-nucleotide "downstream box" which has been shown to improve protein accumulation. Several spectinomycin-resistant lines were obtained for each construct. Successful plastid transformation and homoplasmy were verified by PCR and Southern blot analyses, respectively. Western blot analysis showed a highly significant different accumulation of the recombinant protein with the two vectors, the best results being achieved in LS1-plants. These data confirm that the choice of 5' regulatory sequences can dramatically change the yield of recombinant proteins in the plastome. The precise estimation of ApoA-I expression level in transplastomic plants is currently underway.

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Poster Abstract – 7.07

IMAGE ANALYSIS TOOL FOR VETCH VARIETIES IDENTIFICATION BY MEANS OF SEEDS INSPECTION

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Vicia sativa L., Vicia villosa Roth., seeds, image analysis, varieties identification

Vicia sativa L.(common vetch) is an annual cleaning crop mainly used in rotation with durum wheat, either for seed and forage yield, in purity or undercropping with other species, well adapted to the hot-dry climate and poor soils of Southern of Italy.

Cultivar identification is done on distinctive traits relieved at variety registration following official protocols (UPOV).

The peculiar traits of seeds of some vetch varieties allowed a clearly distinctiveness mainly on some seed features (shape, size, ground color of testa; brown and black ornamentation. Although seed identification process by specialized technicians is possible for some vetch varieties, it is slow and somewhat subjective giving results which may be difficult to quantify both for business and technological implications. Therefore it is important economically and technically to implement repeatable and quick automated methods to identify and classify seeds.

To aid the visual inspection, or replace the human judge in distinguishing different varieties of common vetch, this work presents the development of an image analysis based tool, for the seeds identification of examined varieties.

Colour, shape and size of seeds of nine cultivars of common vetch (*Vicia sativa* L.) and one of hairy vetch (*Vicia villosa* Roth) were measured on acquired images by flatbed scanner, using a specifically developed macro, based on image analysis library KS-400 V 3.0 (Carl Zeiss, Germany).

Statistical classifier to identify the cultivars was obtained using the image analysis data elaborated with the Linear Discriminant Analysis algorithm (Venora et.al 2007; 2009). The performance of classifier was 99.2 % in the training set and 88.20% in the test set respectively. The macro, called *Vetch.mcr*, works quickly and accurately in a cheap system.

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Poster Abstract – 7.08

COMPARATIVE GENOMICS AND EVOLUTIONARY ANALYSIS OF CHLOROPLASTS

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comparative genomics, chloroplast evolution

It is generally accepted that chloroplasts have arisen form an internalized cyanobacterial ancestor through at least two (primary and secondary endosymbioses) events. The chloroplast genome consists of circular double stranded DNA molecules of 110–200 kb size, containing RNA genes and a number of protein coding genes, ranging from about 100 in plants and green algae to 150–200 in non-green algae. The protein-coding genes are involved in the chloroplast expression and translation machinery, or related to photosynthesis. The chloroplast genome of most plants harbors two large inverted repeats (IRs) of 6–76 kb that divide it in one large and one small single-copy region (LSC and SSC, respectively)

The non-recombinant, uniparentally inherited nature of organelle genomes makes them potentially useful tools for evolutionary studies. However, detecting useful polymorphism is difficult due to the slow mutation rates of chloroplast genes and the attempts to reconstruct plant evolution using sequence-based analyses of genes have proven particularly difficult. Furthermore, it is now clear that many of the proteins needed for plastid functions, are encoded by genes located in the nucleus acquired during evolution by Endosymbiotic Gene Transfer (EGT) from the cyanobacterial genome.

The availability of fully sequenced chloroplast genomes opens up the possibility to perform a phylogenetic reconstruction and to shed some light on the genome evolutionary patterns. Therefore, the aim of this work was to analyze the completely sequenced chloroplast genomes in order to (i) carry out a comparative evolutionary analysis of chloroplasts, (ii) a comparative analysis of chloroplast *vs* cyanobacterial genomes.

All the 136 available chloroplast genomes from land plants, green and non-green algae and protists (i. e. the chromatophore of *P. chromatophora*) and the genome of *Nostoc punctiforme* were retrieved form GB. A recently developed bioinformatic tool, Blast2Network, was used to perform the analysis.

Despite the high synteny exhibited by the different chloroplast genomes, the analysis revealed interesting discontinuities of similarity along the generally accepted Viridiplantae phylogeny. Whilst the chloroplasts of some basal land plants (i. e. *P. nudum*, *A. formosae*) confirm their ancestry to the Angiosperm clade, the chloroplast of *P. patens* did not show a high similarity (at least at high threshold value) either with green algae or with angiosperm chloroplasts. Particularly interesting is the absence of high degree of similarity between the available gymnosperm chloroplast and the other tracheophyte, marking a putative incongruence in the phylogenetic position of this group.

The use of Blast2Network enabled also to compare the distribution and the dimension of paralogous gene regions within the same chloroplast genome; large IRs, containing protein coding genes, are present in some green and red algae and diatoms (possibly for recent recombination events), while are largely absent in land plants. The construction of high similarity protein cluster enables also the identification of chloroplast conserved “cores”. Preliminary comparative analysis of chloroplast and *N. punctiforme* chromosome suggests a unequal contribution of the bacterial genome to different plastids, showing possibly the imprints of primary and secondary endosymbioses.

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Poster Abstract – 7.09

A HORIZONTAL GENE TRANSFER AT THE ORIGIN OF PLANT PHENYLPROPANOID METABOLISM

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Phenylpropanoid metabolism, Phenylalanine Ammonia Lyase, land plants, Horizontal Gene Transfer

The pioneering ancestor of land plants (Embryophytes) that conquered terrestrial habitats around 500 million years evolved from the Charophyceae, a small group of freshwater green algae, differentiating afterwards from simple structure (Bryophyte) to complex organisms showing an extraordinary array of organs and tissue systems (vascular plants). However, early land plants had to face a harsh environment characterised by important stresses including desiccation, UV radiation, and microbial attack. Beneficial associations between fungi (mycorrhizal symbioses), and soil bacteria (N_2 fixing), might have greatly helped the first stages of phototrophs terrestrialization.

A key step during plants colonisation of land and diversification was represented by the origin and evolution of the phenylpropanoid pathway; the complex set of phenylpropanoid compounds are in fact involved in many stress response pathways (pathogens, grazing, ROS scavenging, UV screening, etc) as well as in other fundamental traits such as biosynthesis of lignin, the structural polymer able to guarantee stem rigidity and xylem (water conducting tissue) formation.

Despite its importance, the origin and evolution of the phenylpropanoid pathway, as well as the first advantageous physiological roles of its products are still unclear.

Phenylalanine Ammonia Lyase (PAL) is responsible for the first committed step of plant phenylpropanoid pathway. Although the complete phenylpropanoid metabolism appears to be a specific and ubiquitous feature of land plants, PAL homologs have been identified and characterized in fungi such as *Aspergillus oryzae* - whose genome shows to contain several phenylpropanoid pathway genes – and, although phenylpropanoids are largely absent in prokaryotes, in *Streptomyces maritimus* and *Photorhabdus luminescens* where they are involved in the production of antimicrobial compounds. A PAL homologue was also recently discovered in two cyanobacterial species of the order Nostocales

PAL is homologous to Histidine ammonia lyase (HAL), which is involved in histidine degradation and it is present in prokaryotes and eukaryotes. It is thus commonly suggested that PAL evolved from HAL in fungi and plants.

To shed some light on these issues, we have carried out an extensive phylogenetic analysis of PAL and HAL homologs.

The phylogenetic data lead us to propose a new evolutionary scenario involving two horizontal gene transfers: PAL originated in soil bacteria with an antimicrobial role, and was transferred (possibly from Nostocales species) very early to fungi *via* lichen-like symbioses and

then to early land plants *via* ancient arbuscular mycorrhyzal symbioses, enabling the further development of the phenylpropanoid pathway and the radiation of plants on land.

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Poster Abstract – 7.10

AFFYCUSTOM, A VERSATILE R SCRIPT FOR CUSTOMIZATION OF GENECHIP DEFINITION FILES

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Affymetrix, GeneChip, microarray, heterologous hybridizations, Custom Probesets

Standardized microarray platforms for gene expression analysis, such as Affymetrix GeneChips, are available off-the-shelf for a limited set of plant and animal species. For instance, no standard microarrays are available for relevant species, e.g. potato and sheep, and wild relatives of other major species that are relevant in various field of research as studies on crop resistance to environmental stresses. Nevertheless, inspecting the whole trascriptome of these species would be an opportunity to elucidate the molecular mechanisms involved in various biological processes of broad interest.

In Affymetrix GeneChips, genes are interrogated by multiple probesets, each composed of 11 or 16 oligonucleotide probes matching different portions of a specific sequence. The association between probesets and genes is encoded in the Chip Definition File (CDF), which defines the physical design of the microarray and contains the sequences linking the oligonucleotide probes to the interrogated transcripts. Since the association between probes within a specific probeset is not a physical association on the microarray, original probeset definitions can be modified into custom-CDFs producing novel grouping of probes into probesets. The definition of custom-CDF opens the possibility to reorganize the probesets of a GeneChip for a different species into custom-probesets including only the probes matching a different genome for which a set of expressed sequences is available but the corresponding GeneChip is not. In principle, once defined the appropriate custom-CDFs, standard off-the-shelf Affymetrix GeneChips can be utilized for heterologous hybridization on a broader set of organisms.

The custom redefinition of probesets has already been applied to various mammals GeneChips with the aim of refining probeset annotations according with up to date genomic sequences. Nevertheless, a simple and user-friendly procedure to obtain custom-CDF files from any user-defined set of sequences is not available yet.

This work presents AffyCustom, an R script to generate custom-CDFs. The script requires as input i) a FASTA formatted file containing transcript sequences for the organism of interest and ii) the Affymetrix GeneChip that will be used as the basis for building custom probesets definitions. The procedure compares the probe sequences of the selected GeneChip with the user provided transcript sequences, adjusting the mismatch tolerance through few parameters. The CDF customization can be performed on standard desktop computers since AffyCustom exploits the memory-efficient sequence matching algorithms of the Bioconductor project (www.bioconductor.org). End users can easily adopt the custom-CDFs since they are compliant

with GeneChip compatible software, including both open source and commercial software. AffyCustom allowed generating custom-CDFs for two species lacking a specific GeneChip, i.e. sheep and potato, directly from standard commercial GeneChips, e.g. bovine and tomato respectively. The availability of these custom-CDFs opens the possibility of performing expression analysis for a variety of organisms having a related species with a specific GeneChip, as using bovine and tomato microarrays for the transcriptional analysis of sheep and potato species, respectively.

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Poster Abstract – 7.11

GENOMIC POLYMORPHISM UNCOVERED BY A SYSTEMATIC APPROACH BASED ON β-TUBULIN GENE SEQUENCE ORGANIZATION

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tubulins, intron length polymorphisms, genetic diversity

One of the main task in plant breeding is the identification of germplasm containing genes that could improve the performance of currently established cultivars. In the past few years, molecular markers, in association with more powerful statistical models, have been applied to genetic analysis and breeding of several crops. Moreover, advances in molecular biology have allowed the development of rapid, sensitive and specific screening methods to study genetic diversity and relatedness among plant species and varieties.

One recently developed, useful genotyping method is based on intron length polymorphisms present in members of the plant β-tubulin gene family (cTBP). The genomic organization of these genes, that encode proteins of relevance for growth, is such to allow a multiple approach for detection of genetic diversity. The cTBP method for plant genotyping is fast, reproducible, transferable among species, capable of recognizing single components in heterogeneous mixtures and doesn't require preliminary knowledge of the target genome. In addition to the classic cTBP method, we are now introducing a new approach that uncovers gene-sequence variability in the 5'upstream region of the beta-tubulin genes. Applied on different cultivated plants, this AFLP-like technique holds the potential to become a new powerful DNA fingerprinting tool for studying genetic relationships and diversity.

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Poster Abstract – 7.12

TOWARDS THE CONSTRUCTION OF A HIGH DENSITY GENETIC LINKAGE MAP OF WHEAT CHROMOSOME 5A

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genetic map, wheat, chromosome 5A

A high density genetic map is needed for anchoring BAC contigs during the construction of a physical map and for DNA sequence assembly. The International Wheat Genome Sequencing Consortium is dedicated to the development of physical maps of individual chromosomes as the first step towards the whole genome sequencing of hexaploid wheat. To undertake this challenge for wheat chromosome 5A, we rely on several mapping populations and different parallel approaches for marker development. Three mapping populations are being used: 1) a Chinese Spring x Renan (CSxR) F2 population; 2) an F2 population derived from CS x CS-*Triticum dicoccoides* disomic substitution line for chromosome 5A and, 3) a RIL (Recombinant Inbred Lines) population derived from Langdon (LDN) x LDN-T. *dicoccoides* disomic substitution line for chromosome 5A. For marker development, a Diversity Array Technology (DArT) platform specific for the short and long arms of wheat chromosome 5A has been established using DNA from flow sorted chromosomes, and includes more than 6000 wheat probes. So far, this array is under hybridization with one population (CS x R) while the other two populations are in the pipeline. Besides the DArTs, a set of SSR, RFLP-derived and EST-derived PCR-based markers, specific for 5AS and/or 5AL chromosome arms have been selected from databases and literature. After the assignment to chromosome 5A, performed using CS deletion and aneuploid lines, the markers are being tested for

polymorphism between the parents of the three mapping populations. Polymorphic DNA fragments, specific for 5A, will be mapped in the available population(s). The resulting genetic linkage map of the wheat chromosome 5A will be presented.

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Poster Abstract – 7.13

SINGLE NUCLEOTIDE POLYMORPHISM GENOTYPING IN DIPLOID AND TETRAPLOID WHEAT B GENOME

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single nucleotide polymorphism (SNP), genotyping, polyploidy, wheat

Wheat was domesticated about 10,000 years ago and has since spread worldwide to become one of the major crops. Wheat species form a classical polyploid series of diploid, tetraploid and hexaploid species. According to the most accreditated hypothesis, tetraploid wheat *Triticum turgidum* (genome formula AABB) evolved via hybridization of two diploid species closely related to *T. urartu* (genome formula AA) and *Aegilops speltoides* (genome formula SS, where S is similar to B). Polyploidization has been crucial in the establishment of wheat as a major crop, consequently an understanding of this process is crucial both for gain a better insight into the evolution of wheat and for the development of novel and efficient breeding strategies.

In genetically diverse diploid populations, the recurrently occurring allopolyploids generated a bridge for gene flow from the parental diploids to a nascent allopolyploid species. This gene flow broadened the gene pool of the nascent allopolyploid and facilitated its adaptation and evolutionary success as a new species. Although it is becoming evident that most polyploid species have originated recurrently, only fragmentary data on the contribution of the gene pools of the diploid parents to the gene pool of a polyploid species are available.

Recent studies on the A and D genome of wheat were made to address the phenomenon. Here we present our preliminary data on single nucleotide polymorphism in the B genome. In particular, 56 pairs of B genome specific primers, 8 for each of the 7 chromosomes, were designed from the wheat SNP database to analyze 200 genotypes belonging to 40 accessions (5 individuals per accession) of geographically distant diploid BB genome and 200 genotypes belonging to 40 accessions (5 individuals per accession) of geographically distant tetraploid AABB genome. Primers were tested among randomly chosen genotypes and amplicons were subjected to Sanger sequencing in order to discover novel SNPs specific to the available accessions. Currently we have been obtaining promising data from the preliminary sequencing analysis. Twenty-three sequences were compared among the genotypes and were able to discover approximately 293 SNPs and 21 indels which is comparatively higher with respect to wheat SNP database; 116 SNPs and 20 indels for the studied 23 sequences. Sequencing analysis of the remaining 33 sequences are in progress of identifying novel SNPs. The perspective is to utilize advanced genotyping technologies, henceforth already established SNPs along with newly discovered SNPs will be used for high-throughput characterization of our accessions.

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Poster Abstract – 7.14

GENERATION OF A NOVEL ALLELIC SERIES OF STARCH BRANCHING ENZYME (SBEIIA) BY A TILLING STRATEGY

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starch branching IIa, high amylose starch, TILLING, High Melting Resolution, wheat

Reserve starch represents the main component in wheat endosperm, whose composition strongly influences the quality and nutritional value of wheat-based food products. A new challenge to develop novel wheats has been recently identified in the manipulation of starch composition. The modulation of amylose/amylopectin ratio greatly affects wheat flour nutritional and processing properties. Currently many efforts focus on the objective of increasing the amylose fraction in cereal starches. Higher amylose content correlates with an increased amount of "resistant starch", a particular starch fraction, considered to have beneficial effects on human health lowering the risk of important diseases .

The two starch components are synthesized from a common substrate, ADP-glucose, by two distinct pathways. Amylose production involves one enzyme – Granule Bound Starch Synthase I (GBSSI) - whereas three classes of enzyme, known as starch synthases (SSs), branching (SBEs) and debranching enzymes (DBEs) take part to the synthesis of amylopectin.

Starch branching enzymes IIa are one of the main targets to increase amylose content in cereals. Recent studies, in bread (Regina *et al., 2006*) and durum wheat (Sestili *et al.,2009*) have shown that the RNA interference technology can result efficiently in the loss of SBEIIa functionality, leading to a drastic change of the amylose/amylopectin ratio compared to the wild type.

The TILLING -Targeting Induced Lesions In Genomes- represents an effective reverse genetics tool for the production of novel allelic variants in valuable agronomic traits. In TILLING the combination of traditional chemical mutagenesis with high-throughput detection methods of point mutations has originated an attractive strategy both for functional genomics and breeding applications overcoming limits associated with transgenic issues.

In this work an EMS mutagenised population of bread wheat has been analyzed for the identification of SNPs (single nucleotide polymorphisms) of interest in the three homoeoalleles *wSBEIIa* located in A, B and D genomes.

Targeted genic regions of the three *SBEIIa* genes have been analyzed in approximately two thousand wheat lines by High Resolution Melting technology, based on the comparison of the melting behaviours of the PCR fragments analyzed. Forty eight mutant genotypes have been characterized for *wSBEIIa-A* and more than ten for *wSBEIIa-B* , confirming a mutation frequency of 1 SNP for each 40 kb associated to the TILLING library. All the mutations described are localized in the coding region.

Worthy of note two are SNPs causing the truncation of the gene product, localized respectively in the exon IX and exon XIII, that have been identified for *SBEIIa-A* gene and one analogous mutation, localized in the exon VI, for *wSBEIIa-B* gene. For all three homoeologues

different mis-sense mutations causing the change of conserved residues located in catalytic domains of SBEIIa enzymes have been identified. Combining those mutations believed to affect enzymes functionality will be carried out to develop novel wheat genotypes with a highly increased amylose content.

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Poster Abstract – 7.15

GENETIC VARIABILITY IN THE PROMOTER REGIONS OF PROTEIN DISULFIDE ISOMERASE IN DURUM WHEAT AND ITS WILD RELATIVES

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Protein Disulfide Isomerase (PDI) promoter, wheat, variability analysis

Protein disulphide isomerase (PDI) is an oxidoreductase enzyme abundant in the endoplasmic reticulum (ER). In plants, PDIs have been shown to assist the folding and deposition of seed storage proteins during the biogenesis of protein bodies in the endosperm. The cloning and sequencing of the genomic and cDNA sequences of three homoeologous genes encoding typical PDI, located on chromosome group 4 of bread wheat, and their promoter sequences have been reported previously. The three genomic sequences showed a very high conservation of the coding region and of the exon/intron structure. The comparison of PDI gene sequences of wheat, rice and Arabidopsis showed a significant conservation of the exon/intron structure across the three species. The RT-PCR expression analysis of the homoeologous genes in cultivar Chinese Spring showed an overall constitutive expression in all analyzed tissues, (roots, leaves, spikes and caryopses), with the highest level of expression in the early stages of developing caryopses. However, the three homoeologous genes exhibited slightly different expression patterns; the expression was higher in spikes for *TaPDI-4A*, in roots for *TaPDI-4B* and in leaves for *TaPDI-4D*. The promoter sequences of the three homoeologous PDI genes possessed some regulatory motifs typical of genes with endosperm specific expression and consistent with their putative regulatory function.

The final aim of our study is to determine the variability extent in terms of number of SNPs and indels of the promoter sequences within and between some wild and cultivated species of wheat. The first effort consisted in designing specific primers targeting the proximal 700 bp of the 4A or 4B PDI promoter regions in order to amplify and compare them in two diploid [*Triticum uratu* (AA) and *Aegilops speltoides* (BB)] and three tetraploid [*Triticum dicoccum* (AABB), *Triticum dicoccoides* (AABB) and *Triticum durum* (AABB)] species of wheat. A total of 200 individual plants were analysed, consisting of five accessions and eight plants per accession for each of the five species.

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Poster Abstract – 7.16

GENOMIC STRUCTURE AND SINTENY CONSERVATION TOWARDS RICE GENOME OF GENES ENCODING PROTEINS OF THE PROTEIN DISULFIDE ISOMERASE FAMILY IN WHEAT

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

Protein disulfide isomerase or typical PDI is the most prominent member of the PDI family, which includes a number of related PDI-like proteins. In the lumen of the endoplasmic reticulum (ER) it catalyzes the formation, reduction, and isomerization of disulfide bonds in newly synthesized secretory proteins, The plant PDI family includes eight phylogenetic classes, whose components differ for number and position of the active thioredoxin-like sites (CGHC) and for presence/absence of other domains (such as inactive thioredoxin-like domains) and of the KDEL signal of retention in the ER. Plant PDIs have been shown to be involved in the folding and deposition of seed storage proteins. In wheat the potential involvement of PDI and PDI-like proteins in the formation of intra and inter-molecular disulfide bonds makes their study particularly interesting, in fact flour quality is strongly affected by the presence of high molecular weight aggregates. We have reported the cloning and characterization of genomic, cDNA and promoter sequences of the typical PDI and of eight full length cDNA sequences coding for PDI-like proteins in wheat. Phylogenetic analysis assigned the PDI and PDI-like sequences to the eight phylogenetic groups identified in plants, confirming that at least one gene had been cloned for each phylogenetic group.

In this study we report the isolation of the genomic sequences for the eight novel PDI-like genes. The comparison of the genomic sequences of the wheat PDI-like genes with those of their orthologous genes of rice and *Arabidopsis* showed a high level of conservation of their structural features. Moreover, their chromosome location through aneuploid Southern analyses showed a close syntheny with the corresponding rice genes. Finally, the expression patterns of the eight PDI-like genes were analysed by quantitative real-time RT-PCR in a set of 29 samples, including different tissues, developmental stages and temperature stresses.

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Poster Abstract – 7.17

THE IMPACT OF INTRA AND INTER SPECIFIC NUCLEAR-CYTOPLASMIC INTERACTION ON THE REGULATION OF CENTRAL METABOLISM IN WHEAT

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alloplasmic lines, wheat, nuclear-cytoplasmic interaction, metabolite profiling, gene regulation

Breeding programs relies on the understanding of the regulation of traits. Phenotypic diversity depends on the regulation of genes at transcriptional and post transcriptional level. Thus the interaction between nucleus and cytoplasm is a key component in this multileveled regulatory network. Nonetheless, in spite of the importance of nuclear-cytoplasmic crosstalk, the mechanisms by which it affects phenotypic traits remains largely unknown. In an effort to gain further understanding on the regulation of traits in crops, we have explored the role of intra and interspecific cytoplasm regulation of central metabolism, storage reserve accumulation and gene expression in wheat. Three different alloplasmic lines were used in this work to investigate the effect of *H. chilense*, *Ae. uniaristata* and *Ae. squarrosa* cytoplasm on nuclear-cytoplasmic interaction in common wheat. Earlier works showed a clear effect of cytoplasm in these lines on agronomic traits such as anthesis timing, yield and plant height. In the present study we employed GC-MS based metabolite profiling and is in progress the Affymetrix Wheat Gene-Chip® expression profiling to study the set of alloplasmic lines at the molecular level. Our preliminary results show a clear effect of cytoplasm on central metabolism of grain and leaves. We also show a differential metabolic response to low and highlight conditions as well as species associated cytoplasmic influence on metabolite content and transcript levels.

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Poster Abstract – 7.18

BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF "TIMILIA" AND "RUSSELLO" DURUM WHEAT SICILIAN GENOTYPES

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durum wheat, SDS-PAGE, microsatellites

Durum wheat landraces cultivated in Sicily in the first half of the last century, represent a rich source of germplasm particularly suitable to Mediterranean conditions. Some of these ecotypes as 'Timilia' and 'Russello' are still cultivated in limited areas of Sicily and used to produce typical local bread. 'Timilia'grain flour is used for the preparation of the handmade bread from Castelvetrano ('Pane Nero di Castelvetrano'), widely diffused in the western area of Sicily. Whereas, Russello landrace is used for the preparation of bread of Iblea's areas.

Biochemical analysis of storage proteins for the determination of high (HMW) and low molecular weight (LOW) glutenins subunits were carried out in both landraces, by means of SDS PAGE method. A total of 13 microsatellite primer pairs were tested for Timilia and Russello landraces characterization using the Genetic Analyzer 3130 Applied Biosystems and Gene-Scan 4 software.

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Poster Abstract – 7.19

ASSESSING ESSENTIAL DERIVATION IN DURUM WHEAT BY MEANS OF AFLP AND SSR MARKERS

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EDV, durum wheat, genetic similarity, AFLPs, SSRs

The concept of essential derivation refers to cases where new varieties are developed by means of practices such as mutagenesis, genetic engineering, selection within a variety and repeated backcrossing from protected varieties, without a genuine breeding effort. Its implementation entails the definition of a threshold value of genetic conformity allowing to identify cases of suspected derivation. Molecular markers have been indicated as the most appropriate tools for estimating genetic similarities among varieties. Thresholds and technical protocols for EDV identification have been already proposed for a few species, but information is lacking for durum wheat.

Genetic similarity was assessed on a sample of 60 entries, including parental varieties and their F8- or F9-progenies characterised by different levels of relatedness. Molecular analysis was carried out using 14 AFLP primer combinations (*Sse*8387+2-*Mse*I+2) and 105 SSR loci evenly distributed in the durum wheat genome. In both cases, the EDV threshold was defined according to the "tail principle" as the 95 percentile of the distribution of Jaccard similarities among independently developed progenies. Both approaches allowed to identify all cases of tightly related lines separated in advanced generations (F7-F8). Moreover, a good overlap was observed between the cases of suspected essential derivation evidenced by the two marker classes. Due to their high multiplex ratio, at present AFLP seem to be preferable to SSR markers to assess cases of suspected derivation.

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Poster Abstract – 7.20

SEGREGATION DISTORTION FOLLOWING INTROGRESSION OF *THINOPYRUM PONTICUM* DNA INTO DURUM WHEAT

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wheat-alien introgression, gametophytic competition, gametocidal genes, male germline

Segregation distortion (SD) is the deviation of observed genetic ratios from the expected Mendelian ratios of a given genotypic class within a segregating population. Genetic elements that cause SD may be potent evolutionary forces, particularly in terms of species differentiation and genome restructuring. Distorted segregation ratios may result from gametophytic competition, resulting in preferential fertilization or abortion of gametes or zygotes, with most systems affecting the male germline. Another generalization of SD studies is that the underlying mechanisms do not distort meiosis *per se* but rather alter the products of meiosis, often, but not exclusively, by rendering non-functional gametes that do not carry the driving allele(s). Segregation distortion (*Sd*) genes are therefore also referred to as gametocidal (*Gc*) genes.

SD has been observed in a wide variety of organisms, including fungi, plants, insects, and mammals. In plants, genomic regions harboring markers with abnormal segregation ratios have been reported in many crop species, including barley, pearl millet, tomato, rice, maize and wheat. Chromosomes carrying *Sd* genes have been also identified in several wild wheat relatives and their effect revealed upon hybridization with wheat. In most cases such alien chromosomes or chromosome segments are selectively or sometimes exclusively retained in the wheat background. This is the case for various *Aegilops* species and for species of the *Agropyron* and *Thinopyrum* genera. For at least some of these genes, direction and magnitude of the SD effect are determined by the genetic background of the recipient wheat. For instance, the *Sd1* gene, located on *Thinopyrum ponticum* 7AgL arm, proximal to the leaf-rust resistance *Lr19* gene, once incorporated into common wheat through 7DL-7AgL translocations, determined a wide range of effects, from preferential transmission to self-elimination, but also normal segregation of the carrier chromosome, depending on the allelic variation at several wheat "responder" loci. No knowledge is available on the mode of action of *Sd1*.

Among several *T. durum-Th. ponticum* recombinant lines we have previously produced and shown to carry different portions of 7AgL replacing wheat 7AL, normal segregation was observed for chromosomes with up to 28% of 7AgL, containing the alien *Lr19+Yp* genes. On the other hand, a line with 40% 7AgL (R23-1) exhibited reduced transmission of the recombinant chromosome through the male germline. Analysis of F2 and F3 progeny from the cross of R23-1 with different durum wheat varieties showed segregation ratios ranging from normal to highly distorted (always in the direction of self-elimination), due to effects ascribable to the varietal genotype, but with heterogeneity observed also among progeny of the same cross. Although previous mapping data suggested *Sd1* not to be included in the 7AgL segment of R23-1, this possibility cannot be completely ruled out. To investigate possible causes of the observed SD effect and define with more accuracy the location of its driving factor(s), meiotic and post meiotic stages of pollen development have been analyzed in R23-1 as compared to lines with 28% or 23% of 7AgL and their controls.

Preliminary results confirm R23-1 to be the only line presenting various irregularities, mostly affecting post-meiotic stages.

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Poster Abstract – 7.21

IDENTIFICATION, FULL-LENGTH SEQUENCE ANALYSIS AND EXPRESSION OF TRANSCRIPTS ENCODING ALLELIC VARIANTS OF LIPOXYGENASE A IN DURUM WHEAT GRAINS

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durum wheat, lipoxygenase, pasta colour

The yellow colour of pasta products is due to the presence in semolina of carotenoids, mainly free and esterified lutein, and is considered by consumers an important feature. Unfortunately, during pasta making a loss of colour is often observed, probably due to the lipoxygenase (LOX)-linoleate system. LOX catalyses the hydroperoxidation of the polyunsaturated fatty acids containing 1,4-cis, cis pentadiene structures; radicals produced during the intermediate states of substrate hydroperoxidation cause oxidation of carotenoid pigments and hence loss of yellowness in pasta products. Three different LOXs, LOXA, LOXB and LOXC, have been characterized in cereals. Previous findings demonstrated that LOXA accounts for most of the total LOX activity in mature grains. In durum wheat Dubcovsky and coworkers (*J. Cereal Sci*. 2007, 45: 67-77) showed the existence of a duplication of the LpxB1 locus so highlighting the existence of two allelic variants of LOXA gene, namely DQ474240 and DQ474241. They also demonstrated that the deletion of DQ474240 copy is associated with a strong reduction in LOX activity in mature grains.

In order to gain further insights into this point, in this work an analysis was carried out at both molecular and biochemical level on different Italian cvs of durum wheat in order to evaluate the distribution of the DQ474240 and DQ474241 genes as well as the transcript levels of the corresponding expressed sequences, and to relate them with the LOX activity measured in mature grains. The analyses were carried out on two groups of Italian cvs characterized by opposite values of LOX activity: 11 with high LOX activity (HLA) and 6 with low LOX activity (LLA). As expected, genomic analysis confirmed that DQ474240 was present in all the HLA cvs, whereas it was absent in all the LLA cvs; an the other hand, DQ474241 gene was present in all the LLA cvs and in 6 of the 11 HLA cvs. Unexpectedly, a deleted LOXA gene variant was also found in all the LLA cvs. As far as the expressed sequences, two clones were obtained corresponding to DQ474240 and DQ474241 genes, which covered the complete coding sequence of 861 amino residues and showed, at amino acid level, 94% and 95% identity, respectively, with barley LOXA, and to each other of 99%. The transcript corresponding to the deleted variant was also found, whose sequence predicted a protein of 366 amino acids which lacked a central sequence of 495 amino acid residues (from 163 to 657) and, in the shared regions, showed 98% and 95% identity with the full-length sequences corresponding to DQ474240 and DQ474241 genes, respectively. An expression analysis of the three cDNA sequences was carried out in the HLA and LLA cvs. Transcripts revealed the same distribution among cvs than the corresponding genes. The DQ474240 gene was expressed at high levels in all the HLA cvs; contrarily, the DQ474241 gene was found to be expressed at very low levels both in the LLA cvs and in the 6 HLA cvs carrying the DQ474241 gene. As far as the deleted transcript, it was expressed at very high levels in all the LLA cvs.

In conclusion, the full-length cDNA sequences coding for two complete and one deleted variants of LOX A in durum wheat grains is reported here for the first time. Moreover, evidences are reported that high LOX activity is related to high expression of the DQ474240 gene; contrarily, low LOX activity is associated to very low expression of the DQ474241 gene and very high expression of the deleted transcript.

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Poster Abstract – 7.22

ISOLATION OF THE LIPOXA GENOMIC LOCUS BY SCREENING OF A DURUM WHEAT BAC LIBRARY

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durum wheat, BAC library, HMW DNA

Libraries of large-inserts DNA are essential for physical mapping, map-based cloning, identification of molecular markers closely linked to quality traits loci (QTL), gene structure and function analyses in complex genomes.

We prepared a Bacterial Artificial Chromosome (BAC) library from durum wheat cv Ofanto, *Triticum turgidum L. ssp durum,* 2N=4X=28, genome size about 11.200 Mb. Our BAC library is composed of 333.472 BAC clones, arrayed in 869 well plates, with an average insert size of 120kb, for a total coverage of 3.5x wheat genome equivalents and the contamination of chloroplast DNA is 0.3%. About 50% of the BAC clones (147.456) were blotted in four clones bulks onto 24 nylon filters . The filters were screened with an EST deriving from a "totipotent" cDNA library, constructed in the durum wheat cv. Ofanto (Patent N. WO2005003344), similar to the LipoxA gene, coding for lipoxigenase, involved in quality of durum wheat semolina.

Eleven positive BAC clones were isolated using standard protocols for further structural analysis.

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Poster Abstract – 7.23

SET UP OF METHODS TO CHECK THE AUTHENTICITY OF DURUM WHEAT-BASED FOODS BY ANALYSIS OF DNA MICROSATELLITES

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food authenticity, durum wheat chain, microsatellite, quantitative PCR, traceability

Interest in the applications of DNA analysis has increased dramatically over the last decades. In particular, in relation to consumer's expectations regarding food quality, special attention is devoted to DNA analysis to check food authenticity. Durum wheat (*Triticum turgidum* L. Thell. subsp. *turgidum* convar. *durum* Desf. MK.) is the main ingredient of pasta, but it is also used for bread-making all over the Mediterranean area. Currently, Italian law prohibits the manufacture of pasta containing more than 3% soft wheat (*Triticum aestivum* L. Thell. subsp. *vulgare* Vill. MK.) for the domestic market, and in countries where pasta may contain soft wheat it has to be clearly indicated in the label. Besides, sometimes pasta labels claim the use of certain good quality cultivars, and also for some breads from Southern Italy, awarded by Protected Designation of Origin (PDO) at European level, their typicality is related to the exclusive employ of semolina deriving from certain cultivars of durum wheat. As a consequence, there is a need of methods for detecting and quantifying soft wheat adulteration, and for achieving cultivar identification and tracing in durum wheat pasta and bread. The aim of this research has been employing the microsatellite analysis to A) detect soft wheat, or B) distinguish cultivars. For the first purpose, an efficient D-genome-specific repetitive DNA sequence able to detect common wheat was identified. Qualitative PCR experiments were able to assess the presence of common wheat in semolina and durum wheat bread, and Sybr Green real-time PCR was used to quantify the soft wheat adulteration in semolina, with a threshold of 2.5%. An improvement of this method was established by cloning and sequencing the target DNA region, and designing a couple of primers and a dual-labeled TaqMan probe able to quantify soft wheat in pasta. For achieving cultivar identification and tracing, the combined analysis of microsatellite electrophoretic profiles enabled the elaboration of an identification key of monovarietal semolina from the 20 most diffused Italian durum wheat cultivars. Besides, after a previous selection of markers having high polymorphism but, at the same time, characterised by simple electrophoretic patterns, microsatellites were used to check the authenticity of composite semolina used for PDO Altamura bread, derived from the durum wheat cultivars Appulo, Duilio, Arcangelo and Simeto. In the obtained multi-band profile it was possible to distinguish the contribute of the single cultivars required for PDO mark and to verify their presence.

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Poster Abstract – 7.24

THE *hem*L GENE AS A SAFE ALTERNATIVE MARKER IN DURUM WHEAT TRANSFORMATION

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marker gene, wheat transformation, hemL, gabaculine

In recent decades, the development of recombinant-DNA and genetic transformation technologies has allowed hybridization barriers to be overcome, making possible the asexual delivery into crop species of genes of agronomic interest belonging to phylogenetically unrelated sources. The detection of transgenic plants in most crop species requires the use of selectable marker genes and selective agents in order to allow only the cells that have integrated and express the foreign sequence to regenerate into a plant. The commonly employed selectable markers in plant transformation are genes conferring resistance to toxic compounds such as herbicides or antibiotics. However, the presence of these genes in crop plants grown in the field is useless as they serve only in the selection phase, and " unsafe " because of their potential harm to environmental and human health.

A recent alternative proposed as a "safer" marker is the *hem*L gene isolated from *Synechococcus* strain GR6, coding for a mutant form of the enzyme GSA-AT (glutamate-semialdehyde aminotransferase) which is insensitive to the phytotoxin gabaculine. The *hem*L gene was found very efficient in tobacco (Gough et al. 2000) and alfalfa (Rosellini et al. 2007) genetic transformation using gabaculine as selective substance. The *hem*L gene can be a good candidate for a safer selection system as it is present in all plant species and it is involved in one metabolic step only, so that unintended effects of its over-expression in plants are not probable.

The objective of the present work was to test for the first time the selection system based on gabaculine in durum wheat transformation. The efficiency of the *hem*L gene as a selectable marker was compared with a conventional selection system based on the *bar* gene from *S. Hygroscopicus* used as selectable marker gene and Bialaphos as the selective agent. A preliminary study on gabaculine toxicity was conducted on wheat immature embryos and calli, in order to investigate the phytotoxic effects in the callus formation and somatic embryogenesis, and to establish the best concentration to be employed for the transformants selection. A co-transformation experiment was carried out on durum wheat calli by delivering the plasmids pAPCK-*hemL* and pAHC20-*Bar*, carrying the *hem*L and the *bar* genes respectively. After gene delivery, selection was performed separately with the two substances, gabaculine and Bialaphos, for a direct comparison of transformation and selection efficiency. The two selection systems were evaluated during all the stages of transformed plants selection, from callus regeneration to adult plants formation.

In our work *hem*L proved a very efficient selectable marker for durum wheat transformation, in fact it performed as well as the *bar* gene in the selection of the transformed plants. Of the six plants regenerated from gabaculine selection and three recovered from Bialaphos, all integrated the *hem*L and the *bar* genes respectively, thus allowing both markers to recover a 100% selection efficiency. Basing on the results of this work, we can propose the *hem*L gene as a very good safe alternative marker gene for durum wheat transformation.

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Poster Abstract – 7.25

ANALYSIS OF THE METHYLATION DYNAMICS AT THE MAIZE FLOWERING TIME LOCUS *VGT1*

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flowering time, DNA methylation, Zea mays

Flowering time is an important agronomic trait; so far, only a few loci involved in this phenomenon have been described at the molecular level in maize. The *Vegetative to generative transition 1* (*Vgt1*) locus was positionally cloned on chrom. bin 8.05 after its localization as a major QTL (Salvi et al., 2007, PNAS, 104: 11376-11381). *Vgt1* corresponded to an upstream (70 kb) non-coding regulatory element of *ZmRap2*.7, an Ap2-class transcription factor which was shown to influence flowering time. A transposon (MITE) insertion was identified as a major allelic difference within *Vgt1*. One of the hypotheses is that *Vgt1* might function by modifying *ZmRap2*.7 chromatin through an epigenetic mechanism. Therefore, we decided to investigate the methylation state at multiple regions of ca. 250 bp each, within *Vgt1* and the promoter of *ZmRap2*.7. Following digestion with McrBc, an endonuclease that acts upon methylated DNA, real time PCR analyses were performed on genomic DNA from near-isogenic maize lines carrying different combinations of late and early alleles at both loci. DNA was extracted from leaves sampled at 1-, 2- and 3-leaf stages of development. Preliminary results showed a trend of reduction of methylation from the first through the third leaf stage. However, no clear trend was identified when comparing the relative methylation level between the two alleles (N28 and C22-4/Gaspé) across the six target regions at *Vgt1*. The region closer to the MITE insertion showed a constant and very dense methylation level throughout leaf development and for both alleles. To go more into depth, additional genotypes and stages of development are actually being included into the analyses.

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Poster Abstract – 7.26

THE MAIZE *PIN* GENE FAMILY OF AUXIN EFFLUX CARRIERS: EVOLUTIONARY RELATIONSHIP AND EXPRESSION ANALYSIS

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auxin, PIN family, polar auxin transport, Zea mays

The plant hormone auxin (IAA) controls many central developmental processes of plants, including cell division, cell elongation, vascular tissues differentiation, root initiation, apical dominance, and tropic growth responses to environmental stimuli. Auxin is synthesized primarily in meristematic regions at the shoot apex and is then intercellularly transported in a polar fashion to the whole plant. The Chemiosmotic Hypothesis, formulated in the mid 1970s to explain the polar auxin transport (PAT), predicted the existence of specific auxin influx and efflux carriers. Because the chemical properties of IAA suggested that auxin efflux is the limiting step, the isolation of auxin efflux carriers became the main objective of scientists. The molecular characterization of the *pin1* mutant allowed the identification of the first member of PIN-FORMED gene family. Subsequently, seven other genes similar to *PIN1* were found in Arabidopsis genome and PIN proteins have been shown to play a rate-limiting role in the catalysis of auxin efflux from cells, determining the direction of cell-to-cell auxin flow and, as a consequence, creating the auxin gradients that regulate plant development.

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

To identify genes member of the maize *PIN* family we screened several biological databases. Preliminary phylogenetic analysis using the detected ZmPIN proteins plus rice and Arabidopsis PINs confirmed the widening of monocots PIN family compared to dicots one. We identified at least three orthologs of *AtPIN1*, called *ZmPIN1a*, *ZmPIN1b* and *ZmPIN1c* and we mapped them respectively on the chromosomes 9, 5 and 4. In addition we identified two genes closely related to *AtPIN2* (*ZmPIN2a* and *ZmPIN2b*) and a putative orthologs of *AtPIN4* (*ZmPIN4*). As previously reported in rice, also in maize three putative orthologs of *PIN5* (*ZmPIN5*a, *ZmPIN5b* and *ZmPIN5c*) have been isolated, while, at the moment, only one monocot-specific protein (*ZmPIN10*) has been identified. Semiquantitative RT-PCR expression analysis revealed that newly identified *ZmPIN* genes are differentially expressed during maize embryonic, vegetative and reproductive development. To better understand the role of these genes in controlling seed and plant development we are determining their expression patterns by *in situ* hybridization.

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Poster Abstract – 7.27

SHEDDING LIGHT ON THE MAIZE AUXIN ROADS: ZmPIN1 PROTEIN LOCALIZATION STUDIES

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auxin, PIN1, Polar Auxin Transport, GFP, Zea mays

The flow of a signalling molecule across tissues, or even few cells, that is then traduced into differential growth responses in those cells, is a fundamental concept during the development of multicellular organisms. In maize, such as in Arabidopsis, we frequently observed asymmetrical auxin distribution across adjacent cells during crucial stages of growth and development suggesting that the mechanism of auxin mediated morphogenesis is conserved among mono_ and dicotyledonous species.

Polar intercellular auxin flow, thus, provide vectorial information to plant tissues, making it a unique mechanism for transmitting spatial and temporal signals in plant development. Given that the polarity of auxin flow is modulated by changes in the subcellular localization of PIN efflux carriers within each auxin transporting cell, the regulation of PIN protein polarity should be highly controlled, very dynamic and extremely flexible, with the possibility of PIN protein re_localization within a single cell. This plasticity is fundamental to quickly respond to internal and external stimuli and then adapt plant development. In the last years several efforts have been made in *Arabidopsis thaliana* to identify genes regulating the polar targeting of PIN proteins, highlighting a very complex and interconnected regulative network. AtPIN1 basal localization is mediated by the GNOM ADP ribosylation factor/guanine nucleotide exchange factor (ARF/GEF) that functions in endosomal vesicle formation, controlling also AtPIN3 trafficking, while AtPIN2 exocytosis is mediated by SORTING NEXIN1 (AtSNX1) endosomes. The polarity of PIN localization is controlled also by direct phosphorylation of specific PIN residues: the serine/threonine protein kinase PINOID (PID) directly phosphorylates PIN proteins, marking them as apical cargo, while PIN basal localization is regulated by the dephosphorylation catalysed by the trimeric serine_threonine protein phosphatase 2A (PP2A). As a consequence, PIN protein sequence itself contribute to the control of polar PIN polarization thank to the presence of sequence-specific signals.

During our studies on the role of maize *PIN1* orthologous genes throughout embryonic, vegetative and reproductive development, we observed different ZmPIN1 protein localization patterns in different cells or tissues, resulting in differential auxin accumulation patterns. Understand how ZmPIN1 proteins are directed to the plasma-membrane instead to be retained in cytoplasmic vesicles or are directed to distinct sides of the same cells is of outstanding importance to completely understand the role of auxin in controlling cell and tissues polarity in *Zea mays*. Preliminary tobacco protoplasts transformation experiments using ZmPIN1::GFP fusion constructs revealed that the three ZmPIN1 proteins may have different plasma-membrane insertion abilities or, more likely, may be subjected to different regulation pathways. To confirm these results, now we

are analyzing the cell membrane targeting of ZmPIN1::GFP fusion constructs in the homologous system by maize protoplast transient transformation.

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Poster Abstract – 7.28

BRACHYTIC2 AND 3 DOUBLE MUTANT SHOWN AN ALTERED EMBRYO DEVELOPMENT AND PLANT GROWTH

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maize, brachytic mutant, auxin transporter, embryo development, P-glycoproteins

Height reduction has been associated with yield increases in several different crop species. The new varieties, in particular wheat and rice, are shorter, more resistant to storm damage and have an increase grain yield. Brachytic/dwarf varieties have not been exploited commercially in maize, partly because of the excessively severe nature of the original mutant alleles. However similar mutations have been used extensively in sorghum production since the 1950s.

There are three brachytic mutants isolated so far in maize: *brachytic1* (*br1*), *brachytic2* (*br2*) and *brachytic3* (*br3*) that show short stature and gibberellins insensitive phenotype. A maize brachytic mutant of agronomic potential is the recessive mutation *br2*, which results in the shortening of lower stalk internodes with no obvious reduction in other plant organs. *br2* lines exhibit unusual stalk strength and tolerance to wind lodging, the leaves are often darker and persist longer in active green state with respect to wild type plants (Anderson and Chow 1960). The *br2* phenotype is insensitive to treatment with GAs, auxins, brassinosteroids and cytokinins suggesting that the biosynthesis of these hormones is not modified by *br2* mutation.

Among the three brachytic mutants only *br2* was cloned by transposon tagging with *Mu* (*mutator*) element by Multani et al. (2003) and it encodes a putative protein Multidrug Resistant (MDR) class of P-glycoproteins (PGPs) involved in polar movement of auxins. In the past years we isolated a new mutation of *br2* gene and produced double mutant *br2 br3* that showed an altered embryo development and growth suggesting that also *br3* could be involved in the auxin transport.

Furthermore root gravitropic responses are reduced or totally blocked in *brachitic2* and *brachitic3* single mutants. Compared to B73 wild type, *br2* and *br3* mutants seedlings grown vertically and following turned 90°, fail to respond to the changed gravity vector, while seminal roots show correct gravitropic response. These preliminary results suggest the important role of *Br2* and *Br3* genes in controlling plant responses to environmental stimuli such as gravity.

Finally, in this work we report the morphological, genetic and molecular characterization of *br2* and *br3* single and double mutants showing for the first time that these genes are involved in the embryo development.

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Poster Abstract – 7.29

THE LOW PHYTIC ACID1-241 MAIZE MUTATION ALTERS THE ACCUMULATION OF ANTHOCYANINS PIGMENT IN THE KERNEL

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maize, low phytic acid mutation, anthocyanins transporters

Phytic acid (*myo*-inositol-1,2,3,4,5,6-hexa*kis*phospate, or $InsP_6$) is the most plentiful form of phosphate in cereals kernel. Phytic acid is accumulated in the seed, in particular in the scutellum, as a mixed phytate salt of several cations such as potassium, iron, zinc, magnesium, etc. This molecule is degraded by phytase activity during seed germination releasing free phosphate, *myo*-inositol and cations, for seedling growth. Monagastric animals are not able to digest phytate salts that furthermore exhibit an anti-nutritional activity in the feed. For these reasons several breeding programs are developing cereals with lower level of phytic acid compared to traditional cultivar. *Low phytic acid 1* (*lpa1*) mutants in maize have been described so far as the strongest mutations regarding phytic acid biosynthesis. These mutants don't modify the total amount of seed P while reduced phytic acid content associated to a proportionally higher level of free phosphate. So the High Inorganic Phosphate phenotype (HIP), easily determined using Chen's assay, is associated to a *lpa* mutant seeds. Transposon mutagenesis experiments conducted by Shi *et al*. in 2007 demonstrated that *lpa1* gene codified for a Multidrug-Associated-Protein (MRP) named *ZmMRP4* (accession number EF586878).

MRP proteins are transmembrane transporters involved in several functions such as organic ions transport, xenobiotic detoxification, oxidative stress tolerance and transpiration control.

In previous studies several mutations have been isolated in this locus causing a reduction of phytic acid content. In particular *lpa1-241* mutation causes a reduction until 90% of phytic acid associated to strong pleiotropic effects on the whole plant.

In this work we found that *lpa1-241* line, is able to alter the accumulation of anthocyanins in kernel tissues, in genotypes carrying all the genes for anthocyanins biosynthesis.

The anthocyanins are a class of secondary metabolites synthesised exclusively in plants having red coloured tissues, they are water soluble molecules and are present in glycosilated form inside the vacuole where their colour depends in part on the pH of this compart.

In this work we shown that *lpa1-241* mutation enhances the accumulation of anthocyanins in the kernel conferring a blue colour of the scutellum in the *lpa1-241* strongest mutant. Furthermore here we presented genetic, physiological, hystological and molecular data supporting the hypothesis

that the change of anthocyanins colour is due by a defect in their transportation in the vacuole, causing the accumulation of these molecules in the cytosol.

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Poster Abstract – 7.30

TILLING: ALLELE MINING FOR AGRONOMICAL MAJOR TRAITS IN AN ITALIAN RICE VARIETY

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TILLING, rice, functional genomics, reverse genetics, biodiversity

TILLING (Targeting Induced Local Lesion IN Genomes) is a reverse-genetics approach combining chemical mutagenesis with a sensitive DNA screening-technique to identify point mutations in target genes. A TILLING rice population (RICETILL) was developed from an Ethyl Methan Sulphonate (EMS) seed treatment of the rice variety Volano. The Volano cultivar was chosen as being representative of the traditional rice quality and for its relevance in ongoing breeding programs in Italy. This genetic resource was created from a starting population of 20.000 EMS-mutagenized seeds from which 1862 M2 fertile lines were obtained. From each line leaves were sampled and DNA extracted with an automated method. Five candidate genes relevant for responses to grain quality, abiotic stress, plant architecture and flowering time were chosen for investigation: Sd-1(Semidwarfing Gene), PCS -phytochelatin synthase (targeted to heavy metals incorporation into the grain), Waxy (GBSS Granule-Bound Starch Synthase for the grain amylose content), SNAC1 (related to the plant responses to drought tolerance), Hd-1 (control of heading date).

The molecular screening performed on the population for mutations in the M2 DNA samples, was performed at the PTP Genomics Platform with two methods: FLUOTILL analysis of 8- to 12-fold DNA pools and MULTISEQ with 2x DNA pools.. The RICETILL population, although developed for reverse-genetics purposes, is also suitable for forward-genetics analysis and is being tested to identify variants in specific traits important for rice breeding as plant height, resistance to blast, flowering time, amylose content, panicle size. This latter part is described in detail in the poster Cavigiolo S. et al., Obtaining new genetic resources for rice breeding from an EMS-mutagenized population.

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Poster Abstract – 7.31

OBTAINING NEW GENETIC RESOURCES FOR RICE BREEDING FROM AN EMS-MUTAGENIZED POPULATION

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mutagenesis, ethylmethane sulfonate, rice, breeding

Chemical and physical mutagenesis have been used to increase genetic variability in crop plants. In rice (*Oryza sativa L.*), more than 430 new varieties were derived from rice mutants by applying different mutagenic agents (FAO/IAEA Mutant Variety Database). Chemical mutagens (EMS, DEB and sodium azide) and irradiation (Gamma rays, X rays and fast neutrons) have been widely used to induce a large number of functional variations in rice and others crops. Between chemical mutagens, the alkylating agent ethylmethane sulfonate (EMS) is the most commonly used in plants as it causes a high frequency of nucleotide substitutions, as detected in different genomes. Semi-dwarfism (the "green revolution" gene) and earliness are the most frequently described in rice mutant phenotypes. The purpose of the present work is to produce a mutant collection from the Italian rice variety Volano, in order to obtain new lines with different plant height, growth cycle and traits related to yield. Seeds of the cv. Volano were mutagenized by EMS in 2007, at the Department of Biomolecular Sciences and Biotechnology at the University of Milan. Pilot experiments were performed in order to identify the LD50. Subsequently 20,000 rice seeds were treated with a solution of 0.7% EMS. The mutagenized seeds were directly sown in the open field and the M1 generation was grown at CRA – Rice Research Unit, Vercelli. During the growing season the experimental field was visually scored and the off-type plants were removed. At the 3-4 leaf stage, some chlorophyll stripe mutants have been observed. Before the heading stage, each panicle was isolated by a paper bag in order to prevent out-crossing. At the maturity stage, a very high level of sterility (average of 57%) was observed in most of the panicles. At the end of the season the main panicle from each M1 plant was harvested and a total of 1,862 M2 families were obtained. In 2008, seeds from all panicles were sown in the open field. During the growing season, the rice plants were visually scored for the most important traits such as: plant height, panicle length, type of panicle, sowing to maturity interval, grain size and shape. A wide range of morphological variations were observed in the M2 population: short stature, early growth cycle, spontaneous lesion spot, different types of panicles and different level of sterility. The plant height in M2 plants ranged between 35 to 115 cm and 52 M2 plants were classified as dwarf (less than 60 cm height). More than 30 mutants showing an early growth cycle were collected in the M2 population. These plants reached maturity 120-140 days after sowing, much earlier if compared to the cv. Volano (155 days). Some other traits like panicle length, type of panicle and grain dimension were also detected among the M2 plants. According to other studies, several plants developing spontaneous necrotic spots were observed in the population.

All these findings are described and discussed in terms of identification of new traits and induction of biodiversity as an important tool for rice breeding. See also the poster Bruschi G. et al. TILLING: allele mining for agronomical major traits in an Italian rice variety.

This work is performed within the framework of the national project VALORYZA, financially supported by Italian MIPAAF (D.M. 301/7303/06).

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Poster Abstract – 7.32

MOLECULAR MARKERS FOR MOLECULAR ASSISTED BREEDING AND VARIETAL IDENTIFICATION IN ITALIAN RICE

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Oryza sativa L., molecular markers, varietal identification, aroma, amylose content

Fingerprinting methods are a major requirement to track high-quality varieties and protect brand names for the consumer choices. The Italian legislation establishes groupings of varieties and the corresponding trade name for the rice product derived from each group. A trade name must correspond to a single variety of the group within the same package; mixtures among varieties of the same group are not allowed. In the case of specialty rice, such as Carnaroli (for its peculiar quality), Vialone Nano (EU label for Protected Geographical Indication.), and Gange (fragrant rice), the varieties deserve a premium price which is significantly higher than common quality rice in domestic and international market, and packages of milled product must contain the specific variety only. We have developed and validated (ISO9001:2008) a methodology (RICE-ID) based upon a panel of molecular markers as a powerful tool for genetic identification and traceability of Italian rice varieties, applicable not only to non-processed, but also to processed commercial products (e.g. flours). The RICE-ID method enables the unequivocal identification of specific rice genotypes. A dedicated software (RICLASS) was developed to automatically collect and analyze the DNA profiles generated by the DNA fingerprint analysis. The RICE-ID methodology is widely applicable to the analytical tracking of a specific variety during processing of rice-derived food products and to varietal protection and traceability.

Sets of DNA markers were also developped and validated for specific quality traits (e.g. aroma, amylose content). Thee use of these DNA markers allow the selection of genetic materials of interest to accelerate ongoing breeding programs at national level.

The work is performed within the framework of the national project VALORYZA, financially supported by Italian MIPAAF (D.M. 301/7303/06).

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Poster Abstract – 7.33

RICE MARKER-ASSISTED BREEDING FOR COOKING QUALITY

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cooking quality, rice, assisted breeding, waxy, starch synthase IIa

Amylose content and the fine structure of amylopectin are key determinant of processing and eating quality of rice. Parameters such as apparent amylose content (AAC), gel consistency (GC), gelatinization temperature (GT) and the Rapid Visco Analyzer (RVA) profile are commonly used to define eating and cooking qualities of rice, both for theoretical studies and for breeding purposes.

Understanding the genetic bases of starch and cooking properties, and finding PCR-based marker(s) to evaluate the eating and cooking characteristics of rice would contribute to the successful development of lines with the desired cooking quality by means of assisted rice breeding.

The waxy gene (Wx), which encodes the Granule Bound Starch Synthase (GBSS) enzyme, is one of the key genes influencing starch synthesis, and it specifically functions to elongate amylose; while the gene Starch Synthase IIa (SSIIa) is responsible for the varietal differences in amylopectin chain-length distribution, which affects the rice gelatinization temperature.

It has been our aim to find polymorphisms and to characterize alleles of the two genes borne by a large sample of rice varieties and landraces of the germplasm collection of the SIS seed company, representing a cross-section of current and historically important rice Italian germplasm. For this purpose, polymorphisms of a CT-microsatellite, together with a G/T single nucleotide polymorphism (SNP) in the Inton 1, and a C/A SNP in the Exon 6 of the Wx gene were investigated. For the SSIIa gene, a A/G SNP and a GC/TT SNP haplotype , were explored for predictivity of phenotype.

The genotypes have been contemporarily phenotyped for amylose content, while on a subsample of them the Rapid Visco Analyzer (RVA) profiles have been collected.

Most polymorphisms have been derived from literature; for the C/A SNP Ex6 of the Wx gene, the A/G SNP and the GC/TT SNP haplotype of the SSIIa gene, a novel time-saving and improved protocol for assisted breeding have also been developed.

We report here the final results of the study. The alleles, and the allele frequencies carried by all the single genotypes and genotype groups of the collection have been characterized.

Then, the significance of the association between the Wx and SSIIa polymorphisms, the RVA pasting properties and the AAC (%), was assessed through Pearson correlation.

The results showed that the Wx gene polymorphisms play a major role in predicting the starch and cooking properties of rice, and the developed markers can be transferred to routine assisted breeding. Other marker-triats associations are here presented and discussed.

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Poster Abstract – 7.34

FIELD PERFORMANCE, QUALITY CHARACTERIZATION AND ALLELE MINING IN RICE GENOTYPES SUITABLE FOR CULTIVATION IN AEROBIC SOIL

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rice, drought tolerance, breeding, allele mining

Increasing the efficiency of water use in agricultural systems is an essential priority worldwide, including the Mediterranean area. Recent changes of climatic conditions in many areas of the world, have been associated with a drastic reduction of water availability. Rice, as many other crop species, is highly water demanding; rice is also present in diverse agricultural systems including aerobic soils, the so called "upland rice". In China, upland rice genotypes have been successfully introduced into breeding programmes to realize superior varieties characterized by high yield and pronounced tolerance to drought. The improved Chinese germplasm – as it has been at the beginning of the Italian rice history – may represent a powerful genetic resource for the development of Italian varieties suitable for cultivation in aerobic soil under reduced water regime.

The present study, conducted within the three year period of the Eu-funded project CEDROME (INCO-CT2005-015468) reports the final evaluation of a panel of upland and simil-upland rice genotypes from Egypt and China together with Italian varieties and selected lines from CRA-RIS breeding programmes, in aerobic soil conditions. The field experiments were carried out at CRA – Rice Research Unit of Vercelli, Italy. The aerobic soil conditions, also indicated as reduced water regime (RWR) were set up to supplement 1/4 of the water used in the conventional agrosystem.

The experiments allowed to: a) identify the best performing Italian varieties; b) the best performing foreign varieties and better adapted to our environment; c) specific breeding lines derived from our current activities, with interesting performance; d) establish crosses between the best performers identified and undertake a breeding story; e) set up an anther culture system from F1 hybrids for the regeneration of diplo-haploids and establish 336 DH-DT (Drought Tolerant lines). All genotypes were characterized for agronomical (grain yield, harvest index, tiller density, panicle sterility, kernels numbers/panicle and 1000 grain weight), morpho-phenological (plant height, panicle length, flowering/maturity date), phytosanitary and quality traits (amylose content and grain texture). The best performing genotypes, characterized by enhanced tolerance to drought, were subsequently explored at the genomic level, in order to associate their tolerance to allelic variations in candidate target genes. In particular an *OsHox22* gene SNP allelic variation was matched with plant height decrease under drought and other traits in both temperate and tropical *japonica* rice; the allelic variant is present in tropical japonicas as well as in some Italian and

Egyptian drought tolerant genotypes. The same approach is underway for other drought-related candidate genes.

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Poster Abstract – 7.35

ABSENCE OF GLIADIN PEPTIDES TOXIC TO CELIAC PATIENTS IN SORGHUM PLANT SPECIES

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celiac disease, sorghum, prolamin (Kafirin), ELISA, comparative genomics

Celiac disease is a chronic inflammatory disease of the small intestine caused by the ingestion of proline- and glutamine-rich wheat gluten (consisting of the gliadin and glutenin subcomponents) (Sollid 2002). Several different celiac T cell epitopes derived from gliadin proteins have been identified during the last few years, and these epitopes cluster in the proline-rich regions of the proteins (Arentz-Hansen et al 2002).

The 33-mer LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF epitope identified in α_2-gliadin 56-88 (Shan et al 2002) was shown to be particularly important because it is recognized by intestinal T cells of the majority of adult celiac patients (Sollid 2002). The plants that have proteins that damage the small intestine of people with celiac disease are wheat, rye and barley. These three harmful species are members of the grass family and are quite closely related to each another, according to various plant taxonomists. However, not all members of the grass family contain proteins that are toxic to celiac patients. Since corn and rice are safe for celiacs, it is reasonable no expect that members of the grass family that are more closely related to these species (based on taxonomy) than to wheat are likely to be safe. Sorghum, among certain cereal grains, is close enough in his genetic relationship to corn to make it likely that it is safe for celiac patients to eat. There are both protein studies and *in vitro* and *in vivo* challenge of wheat-free sorghum food products that support this conclusion, although the studies are not sufficiently exhaustive to provide more than guidance (Ciacci et al 2007). In the present study we provide molecular evidence on the absence of toxic peptides to celiac patients in this grass species by both HPLC and SDS-PAGE analyses and enzyme-linked immunoadsorbent assay (ELISA) of aqueous/alcohol soluble prolamins (kafirns) from different sorghum varieties. The results of these experiments support the conclusion that sorghum-derived products do not contain proteins that are toxic for celiac patients. Analyses of genes in the recently published sorghum genome sequence provide further support for this assertion (Xu & Messing 2008). Therefore, sorghum can be definitively considered safe for people with celiac disease.

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Poster Abstract – 7.36

FROM GENETIC DIVERSITY ANALYSIS TO DETECTION OF SELECTIVE FORCES, VIA ASSOCIATION ANALYSIS: THE VALUE OF LANDRACE POPULATIONS

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genetic structure, Hordeum vulgare L., linkage disequilibrium, multilocus association, outlier loci

Genetic diversity, population structure and the extent of linkage disequilibrium (LD) are investigated in 11 Sardinian populations of barley (*Hordeum vulgare* L.) landraces, through 134 SSAP markers. The structure of the multilocus association and the presence of outlier loci are also investigated, to determine which sources of diversification have shaped the genetic diversity of the Sardinian barley. Several datasets are used to test the consistency of the genetic diversity analysis results (e.g. datasets without rare alleles or without similar individuals, etc.). The UPGMA analysis shows a major clustering of the barley population landraces according to three main geographic areas of origin. The AMOVA analysis reveals that there is a greater genetic variance within than among the populations. Genetic structure analysis reveals the existence of four main genetic groups, in a genetic subdivision that is only partially explained by geographic patterns. The average LD within landrace populations is low; however, LD levels and the LD rate of decay are population dependent. The multilocus association and outlier detection analyses show that the genetic make-up of this Sardinian barley populations is at least partially determined by selection. Overall, these results demonstrate that these Sardinian barley landraces include valuable material for future breeding studies in Mediterranean environments, while also providing interesting implications with regard to association mapping studies.

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Poster Abstract – 7.37

MOLECULAR VARIATION ALONG AN ALTITUDE GRADIENT IN ETHIOPIAN BARLEY LANDRACES

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Hordeum vulgare, landraces, cline of variation, selection, autocorrelation

To determine the level and pattern of genetic variation in barley (*Hordem vulgare* L.) landraces from North Shewa zone, in the central highlands of Ethiopia, the genetic variability at seven nuclear microsatellite loci was examined. Analysis was carried out on a total of 106 landrace populations sampled in two growing seasons (*Meher* and *Belg*, the long and short rainy season, respectively), across three districts (Ankober, Mojanawadera and Tarmaber), and, within each district, all along an altitudinal gradient (from 1798 to 3324 m a.s.l).

Genetic variation has been ascribed to differences between altitudinal classes (F_{ST} = 0.10) more than between seasons or among districts (F_{ST} = 0.02). The most relevant outcome of the experiment is that altitude level largely overrides geographical distance as main cause of divergence among individual plants. Moreover, results also suggest that the patterns of clinal variation among districts and seasons are inconsistent with a simple model drift and dispersal (seed exchange). They suggested instead a role for historical patterns of colonization, or, alternatively, present-day selective forces acting on some of the SSR analysed.

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Poster Abstract – 7.38

TILLING FOR ROOT MORPHOLOGY MUTANTS IN BARLEY

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Hordeum vulgare, TILLING, forward genetics, reverse genetics, root morphology

Drought is considered the most serious problem for agriculture worldwide. In this context, crops characterized by a more effective use of water will be at an advantage. Among the morpho-physiological traits that have been shown to influence drought resistance, root morphology (shape, depth, size, number of hairs, etc.) plays a crucial role. At the University of Bologna, a TILLING collection of barley (cv. Morex) mutants has recently been produced following chemical treatment with sodium azide (NaN_3). The resource, named TILLMore, can be used for both forward and reverse genetics analyses (Talamè et al., 2008 Plant Biotechnol J 6: 477-485). In this study, four barley genes involved in root development (*Brxl, Rpd*1*, HvExpb*1*, Miz*1) were analysed using the TILLING technology. We identified an average of six alleles per gene corresponding to an extrapolated rate of one mutation every ca. 500 kb. The majority of the detected mutations were CG-to-TA transitions and several of them were missense, implying a change in amino acid sequence.

In parallel, a forward-genetics analysis was performed on a portion of the mutagenized population with the final aim to identify morphological variants at the root level. For this purpose a simple paper-roll approach was performed allowing for the identification of phenotypic variants at the seedling stage. The analysis was completed on ca. 1,000 M3 families and the phenotypic evaluation was repeated only for the putative mutants identified during the preliminary screening. The screening for root phenotype at the seedling stage allowed us to identify a total number of ca. 70 lines with altered root morphology, corresponding to ca. 7% of the families. A more accurate phenotypic characterization of the mutant lines is currently in progress.

Results on both forward and reverse analysis will be presented and discussed.

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Poster Abstract – 7.39

TILLING FOR STARCH METABOLISM MUTANTS IN BARLEY

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Hordeum vulgare, reverse genetics, TILLING, starch metabolism

At DiSTA, University of Bologna, a sodium azide-treated collection of barley (variety "Morex") mutants has been developed for identifying mutations in specific genes using the TILLING procedure (McCallum et al*.,* 2000 Plant Physiol 123: 439-442). The collection, named TILLMore, has already been screened for several (15) genes based on the analysis of DNA pools produced from $M_{2\text{-}3}$ DNA samples, using LiCor and ABI-3730 sequencers (Talamè et al., 2008 Plant Biotechnol J. 6: 477-485). On average, six alleles per gene, corresponding to an extrapolated rate of one mutation every 480 kb, were identified.

A TILLING service for the TILLMore resource is currently available on a cost-recovery basis and/or through collaborative agreements (for details, see www.distagenomics.unibo.it/TILLMore/).

We utilized a TILLING approach to identify mutants for genes related to starch synthesis and degradation in barley. Starch is the major reserve of plants and the primary carbohydrate component in human and livestock diets. Mutants for biosynthetic or regulatory genes of starch metabolism often produce starch granules with abnormal morphological and molecular features that could be of interest also for technological applications.

Molecular screening of TILLMore for mutations has already been completed for five starch-related genes (*Limit dextrinase1*, GBSSI, Bmy1, SSI and SSII) with a total number of 20 mutants identified. Almost all the mutations detected were CG-TA transitions and several (ca. 60 %) implied a change in amino acid sequence and therefore possible effects on phenotype. In four cases, we identified non-sense and splice junction mutations which affect drastically the protein function. A phenotypic characterization of the mutant lines is currently in progress.

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Poster Abstract – 7.40

GENETIC CHARACTERIZATION OF STARCH-BOUND PROTEINS IN AVENA

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oat species, endosperm texture, starch-bound proteins, genetic variability, gene sequencing

Oats (*Avena sativa* L.) possess an extremely soft endosperm and starch granules with unique characteristics. Previous studies indicated that in wheat soft texture is regulated by a class of starch-bound proteins, called puroindolines, that also have emulsifying and antimicrobial properties. In *Avena* a family of starch-bound proteins were identified, structurally similar to puroindolines, which were called vromindolines. These proteins are characterized by the presence of a tryptophan-rich domain and ten cystein residues, like the 2S proteins superfamily, and could be responsible for the extra-softness of the oat endosperm.

The number and molecular weight of the vromindoline components was described by two-dimensional A-PAGE x SDS-PAGE electrophoresis. Genetic variability of these proteins was then explored in a group of 36 cultivated husked and hull-less varieties, and in 14 accessions from oat species with different ploidy levels (2n=14, 2n=28 or 2n=42 chromosomes).

Moreover, some oat cDNA sequences were isolated in a previous study, showing a similarity with the DNA sequences of wheat puroindolines. Specific primers designed on the basis of these sequences were used to amplify the genomic DNA from the oat accessions, and the derived protein sequences were compared in a few species. The preliminary results of the biochemical and molecular characterization of the vromindolines reflected the phylogenetic relationships among *Avena* species suggested in recent studies.

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Poster Abstract – 7.41

FLOW KARYOTYPING AND SORTING OF *DASYPYRUM VILLOSUM* CHROMOSOMES IN SUSPENSION

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flow cytogenetics, chromosome isolation, fluorescent in situ hybridization, wheat improvement

Dasypyrum villosum (*Dv*) has its core distribution in the central-southern Italian peninsular and insular areas, where it strieves in diversified environments (fallows wild grass plant communities in sublitoraneous calcareous sands, non-litoraneous sands or tuffs, scarce soil interspersed to calcareous rocks in semi-arid environments, cold mountainous terrain, disturbed road-sides) expressing several interesting ecological adaptations that shaped its polymorphic genome. The genes for its adaptations (i. e. those for grain storage proteins and resistance to biotic stresses) when introgressed into the wheat genome have made significant wheat trait enhancement.

The introgression of *Dv* genes into wheat can be monitored by *in situ* hybridization and molecular markers but gene isolation and cloning for direct specific transfer has not been achieved.

Flow cytogenetics and sorting could be of use to foster genetic characterization in *Dv* and to allow physical mapping, cloning and then direct transfer of useful genes thus avoiding traditional breeding drawbacks.

In order to obtain the first flow karyotyping and chromosome sorting in *Dv* we have developed a complete system for cell cycle synchronization and chromosome isolation in suspension from fixed root tips. *Dv* seeds were grown in a Hoagland solution supplemented with 1.25 mM hydroxyurea and 2.5 μM amiphrophosmethyl (APM) where root meristems reached a metaphase index of more than 50%. Chromosomes were isolated in suspension after root tip fixation with 2% formaldeyde for 30min and mechanical disruption at 13500rpm for 10sec in LB01 (Dolezel et al.,1989). About $5x10^4$ ml^{-1} intact chromosomes were isolated from 30 fixed root tips.

Theoretical flow karyotype modeling obtained from measured chromosome metaphases was challenged to real flow karyotypes obtained from chromosomes in suspension isolated from double sinchronized root tip cultures showing that only a large chromosome (FISH characterization undergoing) can be sorted from this standard *Dv* ecotype.

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Poster Abstract – 7.42

ISSR AND SSR ANALYSIS OF THE COMMON BEAN (*PHASEOLUS VULGARIS* L.) ECOTYPE "FAGIOLO DI CONTRONE"*

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Phaseolus vulgaris, ISSR, SSR, "fagiolo di Controne"

The common bean *Phaseolus vulgaris* L. ecotype "fagiolo di Controne" is a valuable crop grown near the Calore river, in the province of Salerno (Campania, Italy). In order to reveal genetic diversity both within and among populations of domesticated Italian common bean, several populations of "fagiolo di Controne" and some commercial and typical italian varieties, were investigated by mean of Simple Sequence Repeats (SSR) and Inter Simple Sequence Repeats (ISSR) molecular markers. 22 SSR pair primers chosen from recent literature, specific to a given place in the genome, were tested. They generated from 1 to 9 amplification products and all revealed polymorphic patterns. A total of 63 bands was scored, in the size ranging from 100 to 500 bp.

ISSR analysis was performed with 14 different primers, previously selected on the basis of clarity and reproducibility of bands. They generated from 14 to 26 amplification products and all revealed polymorphic patterns. A total of 219 bands was scored, in the size ranging from 200 to 2500 bp. Amplification profiles of different bean varieties examined revealed a high degree of polymorphism, as generally different band patterns were observed. Low polymorphism was revealed within and among the populations of "fagiolo di Controne". Genetic similarities were calculated according to Jaccard's Similarity Index and used to construct a dendrogram based on UPGMA. The cluster analysis, based on both SSR and ISSR polymorphic fragments, grouped the populations of "fagiolo di Controne" together with "Coco" common beans.

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Poster Abstract – 7.43

NOVEL VARIANTS OF BBI CODING GENES FROM THE LEGUME *LATHYRUS SATIVUS*

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Bowman-Birk inhibitors, Lathyrus sativus, heterologous expression, anticancer activity

Protease inhibitors of the Bowman-Birk family (BBIs) are double-headed inhibitors present in many plant species, particularly in legumes. BBIs have been shown to be capable of preventing or suppressing carcinogenic processes in a wide variety of in vitro and in vivo model systems. The ability of BBIs to inhibit cancer cell proliferation in vitro seems to be related to the presence of an active chymotrypsin inhibitor binding site.

Due to their potential utility, the study of inhibitor variants — differing for protein sequence, inhibition performance and selectivity — might be beneficial in identifying new possible sources of profitable molecules.

With the aim to isolate and characterize new isoforms of genes coding for BBIs, we investigated the genetic structure of these genes in the legume Lathyrus sativus. We isolated a pool of BBI-like genes from L. sativus that, although very similar to each other, show some significant differences. In particular, a comparison between their deduced amino-acidic sequences highlights a putative BBI not yet described in the literature.

In order to perform functional analyses, L. sativus BBIs were heterologously expressed in the yeast Pichia pastoris. After purification of recombinant proteins, their inhibitory activity was evaluated, by means of enzymatic assays using specific substrates for different serine proteases. In a following step purified recombinant proteins will be tested in a series of in vitro assays in order to verify their anticancer activity.

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Poster Abstract – 7.44

PROTEIN AND β-ODAP CONTENT AND THEIR ASSOCIATION WITH YIELD CONTRIBUTING TRAITS IN SELECTED GRASS PEA LINES

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Lathyrus sativus, yield, seed traits, β-ODAP, protein content

Eight selected grass pea (*Lathyrus sativus* L.) lines were evaluated over three different growing seasons (2005-2008) at the experimental field at the 'Sparacia farm' (Cammarata-AG) in Sicily. A randomized complete-block design with three replicates was adopted. 35 viable seeds/m^2 of each line were placed at the end of autumn in rows distant 50 cm. After one summer ploughing, 90 kg ha^{-1} di P_2O_5 were filled during two harrowing before sowing; later two manual weeds control were performed. Harvest was executed at full maturity of pods (end of the spring). Temperature values and effective rainfall for each season were recorded. Information are presented on the variability in storage seed proteins, β-ODAP (β-N-oxalyldiaminopropionic acid) content and their associations with yield, days to flowering, plant height, 100-seeds weight, seed length, seed width and seed thickness. The mean square for lines, environments and G x E interactions were highly significant for yield, 100-seeds weight, days to flowering, plant height and protein content indicating the existence of a wide range of variation between genotypes and that performance of lines was different over seasons. Correlations coefficients and Principal Components analysis were computed as useful tools to summarize the degree of associations between traits and the total variation showed by the tested lines. Results showed for protein content a negative and non significant correlation with most traits. β-ODAP content showed a positive and high significant correlation (r=0.77) only with yield but a negative one with most of the other traits. The first three vectors obtained by the ordination procedure for all traits accounted for 0.86% of total variation. The first component displayed differences in the behaviour of the lines for yield (-0.39), plant height (-0.44), seed length (0.45), seed width (0.33) and ODAP (-0.35); the second component showed different behaviour for protein content (-0.49) and partially for β-ODAP content (0.34); while, 100–seeds weight (0.45), seed thickness (-0.55), and days to flowering (0.60) showed high loadings in the third component. This study suggests that environment, genotype and their interaction affects the protein content and the yield performance; on the contrary, the different sources of variation were found not significant for β-ODAP content. The information here reported may be important to identify the most stable and promising grass pea lines in order to promote the expansion of the crop in sustainable and low–input agricultural systems.

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Poster Abstract – 7.45

SCREENING OF *MEDICAGO TRUNCATULA* COLLECTIONS BASED ON TNT1 MUTAGENESIS

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Tnt1 mutagenesis, Medicago truncatula, leaf, tannin

Medicago truncatula is a main model system for legume species. Genomic tools have become increasingly available in the last years, with the genome sequence being fairly advanced. In particular transposon (Tnt1) based mutant collections are available both in Europe and USA (Ratet et al., 2007; Porceddu et al., 2008; Tadege et al, 2008) . We have established one of the mentioned mutant collection (approximately 2000 R0 plants) which is progressively being screened in forward for visual characters and for the presence of secondary compounds (tannins, saponines). We will report on a few remarkable mutants concerning plant development with a particular emphasis on a mutant that is hampered in leaf abscission. In addition we have performed a screening for the presence of condensed tannins in the leaves/trichomes for about 9000 plants (approximately 12 progenies of 800 R0 lines), whose majority belongs to the collection of the Noble Foundation in USA. We have isolated mutants which are negative for the presence of tannins in the glandular trichomes and we are currently characterizing them.

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Poster Abstract – 7.46

GENETIC TRANSFORMATION OF BARREL MEDIC (*MEDICAGO TRUNCATULA* GAERTN.) USING PLANT-DERIVED TRANSFER DNA (P-DNA)

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forage legume, genetic transformation, GMO biosafety, intragenic plant, ipt gene

The intragenic approach recently developed by Rommens and co-workers (Rommens et al. 2007, Trends Plant Sci. 12: 397-403) allows the production of genetically modified plants carrying only plant-derived DNA and avoided of undesired bacterial sequences. The P-DNA (plant-derived transfer DNA) technology is based on the use of native expression cassettes containing only plant regulatory and coding sequences, inserted into species-specific P-DNAs. The latter are functionally active genetic elements, alternative to the conventional *Agrobacterium tumefaciens* transfer DNA borders.

In the present work, *Agrobacterium*-mediated genetic transformation of the model legume *Medicago truncatula* Gaertn. (M9-10a genotype) was carried out using the pSIM843 vector which harbours P-DNA elements from alfalfa (*Medicago sativa* L.) and the bacterial isopentenyl transferase (*ipt*) gene as backbone integration marker. Undesired events of vector backbone transfer can be detected by monitoring the occurrence of the abnormal *ipt*-shooty phenotype.

Both normal and *ipt*-shooty phenotypes were regenerated following co-cultivation of barrel medic leaf explants with the EHA105-pSIM843 *A. tumefaciens* strain. Molecular analyses are currently performed in order to assess the transfer efficiency of alfalfa P-DNA in barrel medic and the frequency of backbone transfer events.

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Poster Abstract – 7.47

BREEDING FOR EARLINESS/LATENESS IN ALFALFA TO IMPROVE FORAGE QUALITY

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Medicago sativa, stem phenological stage, fiber fractions, NIRS analysis

Stem age and phenological stage in alfalfa (*Medicago sativa* L.) are negatively correlated with stem protein content and positively correlated with fiber fractions content (Kalu and Fick, 1983; Rotili *et al.*, 1997). Besides, we consistently found a positive correlation between dry matter yield (DMY) and earliness in several alfalfa populations and families. The uncoupling of vegetative growth and development is likely to have positive influences on forage quality by reducing the lignification of stems and the senescence rate of leaves. In order to verify these hypotheses, a positive selection for DMY and a divergent selection for the phenological stage at cutting (earliness/lateness) was applied on two different alfalfa populations, SxC, non dormant, and SynT, with intermediate fall dormancy, during two selfing generations. The S_2 selected plants were then manually crossed within the 'early' subgroup to produce simple hybrids (SH) S_2xS_2: seven and ten SHs respectively for 'early' and 'late' subgroup were grown in miniplots 80 cm high x 20 cm diameter (20 plants/plot; density 400 plants m^{-2}) in a randomized block design with 4 replications (4240 plants in total). Irrigation was non limiting.

DMY, earliness and mortality were recorded at individual plant basis along cuttings 2 to 5 in the sowing year, and in cutting 2 in the 1st productive year. Plants exceeding the mean + 0.75*s* within each SH progeny were chosen for the analysis of the fiber fractions by NIRS in cuttings 3 (August) and 4 (September) of the sowing year.

The selection applied in the selfing generations has been effective, as the 'early' and 'late' subgroups were comparable in terms of annual DMY, but significantly differed for earliness estimated by the percentage of plants attaining the reproductive stage at cutting, and the number, length and phenological stage of the reproductive nodes on the two main stems/plant. Interestingly, at the 5th cutting (October) the 'late' subgroup showed DMY and stem height values lower than the 'early' subgroup, suggesting a common genetic control for lateness in flowering and reaction to fall conditions.

The relationships between earliness and stem fiber concentration will be discussed.

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Poster Abstract – 7.48

'FREE-HYBRIDS' IN ALFALFA: ROLE OF HETEROZYGOSITY AND GENETIC STRUCTURE IN HETEROSIS

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free-hybrids, heterosis, SSR markers, heterozygosity levels

Double 'free-hybrids' in alfalfa, a polyploid, allogamous and perennial forage crop, were obtained by crossing in a diallelic scheme six simple hybrids, multiplied for two generations ($2S_2$Syn3), derived from four partly inbred (S_2) constituents (Rotili et al., 1999). The six parental families $2S_2$Syn3, the fifteen double hybrids (DH) and the corresponding synthetic, i.e. the synthetic obtained from the same four S_2 constituents, were studied along 10 cuttings in two years in greenhouse condition. Specific Combining Ability (SCA) source of variation resulted highly significant and larger than General Combining Ability (GCA) component and supported heterosis values of DHs vs the best parent of +45% on average, ranging from +76 to +5%. The investigation at the molecular level of the heterosis found could contribute to dissect the basis of this phenomenon in polyploid species and to identify candidate heterotic groups in alfalfa. For this purpose, the parental $2S_2$Syn3 progenies (13-20 plants per parent), the 15 double hybrids (10 plants per DH, 5 plus and 5 minus for total dry matter yield) and the corresponding synthetic (20 plants, 10 plus and 10 minus for total dry matter yield) have been analyzed by means of 63 SSR markers coming from both *M. sativa* and *M. truncatula*. A part of the SSR markers used have been mapped in *M. sativa* mapping populations (Julier et al., 2003; Sledge et al., 2004).

The heterozygosity and the genetic structure of the 15 DHs will be compared to the parental generation and to the synthetic and the relationship between heterozygosity and heterosis will be discussed.

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Poster Abstract – 7.49

SEXUAL POLYPLOIDIZATION IN *MEDICAGO SATIVA* AFFECTS ADAPTIVE TRAITS AND GENE EXPRESSION

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alfalfa, tetraploidization, microarrays, transcriptomics

Polyploidization is a widespread event in plant evolution that can influence economically important traits. Recent literature demonstrates that modifications in gene expression and/or in DNA sequence occur as a consequence of polyploidization. Our effort is focused on understanding the effects of sexual polyploidization on gene expression and phenotypic traits in alfalfa, a forage legume with tetrasomic inheritance. This information can contribute to elucidate aspects of the selective advantage of polyploidy, and could have practical applications in the improvement of polyploid crops. Two previously characterized diploid (2x=16) plants of the subspecies *Medicago falcata* and *M. coerulea* that produce 2n eggs and 2n pollen, respectively, were crossed, obtaining diploid, triploid (3x=24) and tetraploid (4x=32) progenies from bilateral sexual polyploidization. Diploid and tetraploid progenies of the same parents), allow to separate the effects of hybridization from those of polyploidization on phenotypic traits and gene expression. Three 2x and three 4x progeny plants where selected to investigate polyploidization-induced changes in leaves, by analyzing gene transcription levels using the *Medicago* Genome Array (Affymetrix), contains cDNA-derived sequences of over 61,000 genes from *M. truncatula* (51,000), *M. sativa* (1,800). Comparisons of gene expression levels were made between the parents and between the midparent and each diploid and tetraploid progeny plant. Significant deviations from the midparent transcription level was observed for 189 genes in 4x progenies only, 178 genes in 2x progenies only, and 161 in both 2x and 4x progenies. The comparison between the GO annotations obtained for the 189 polyploidization-affected genes versus the 339 hybridization-affected ones revealed functions that are under- or over-represented between the two groups.

Along with transcriptome, the cytosine-directed methylation patterns was examined in order to study the effect of the polyploidization on whole genome methylation status. Preliminary results show few differences between the parents and the tetraploid progenies compared to diploid and triploid progenies.

Interestingly, polyploid plants flowered earlier and biomass production was faster than in diploid plants. Surprisingly, ovule sterility of the 4x plants was twofold lower, and seed set fivefold higher than that of the 2x plants. Our data indicate that sexual polyploidization can affect important adaptive traits and directly increase fitness. Polyploidization-induced phenotypic changes such as those observed in our materials may have favoured domestication of tetraploid alfalfa.

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Poster Abstract – 7.50

MEDICAGO SATIVA GSA-AT, A NOVEL, PLANT-DERIVED SELECTABLE MARKER GENE FOR GENETIC ENGINEERING

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selectable marker genes, GSA-AT, gabaculine, Nicotiana tabacum, Medicago sativa

The use of selectable marker genes (SMG) of bacterial origin conferring antibiotic or herbicide resistance has been a valuable tool in plant genetic engineering for many years. Consumer concerns and regulatory requirements have stimulated the development of alternative selection systems. In previous experiments, we have estimated that the efficiency of standard marker-free techniques is at present too poor for routine use in alfalfa.

We have previously demonstrated that a mutated form of the *Synechococcus elongatus* hemL gene encoding glutamate 1-semialdehyde aminotransferase (GSA-AT) is efficient a SMG in alfalfa. The enzyme *GSA-AT* catalyses the conversion of glutamate-1-semialdehyde into aminolevulinic acid, a step in the synthesis of tetrapyrrole compounds, including chlorophyll. GSA-AT is irreversibly inhibited by gabaculine (3-amino-2,3-dihydrobenzoic acid). The mutated bacterial gene has a point mutation resulting in the substitution of a methyonine with an isoleucine in the catalytic site of the enzyme, and a three aminoacid deletion close to the N terminus.

With the aim to develop a plant-derived, non-antibiotic, safe, SMG, we cloned and sequenced the *Medicago sativa* (*GSA-AT*) cDNA**.** Then we point-mutated the gene by PCR, so to reproduce the bacterial methyonine to isoleucine substitution.

This mutated *GSA-AT* gene (*MsGSA-gr*) was assessed for the ability to confer gabaculine resistance in *Nicotiana tabacum* and *Medicago sativa* transformation *via Agrobacterium tumefaciens*.

Two transformation experiments were performed for both species. In tobacco, 46,5% and 40,3% of the leaf explants produced green shoots in the presence of 30 μM gabaculine. In alfalfa, the observed percentages have turned out higher: 92,3 % of the explants produced green embryos. Moreover our preliminary data indicate the complete absence of escapes in both species.

The very good results achieved with tobacco and alfalfa transformation suggests that this new SMG can be applied with success to other plant species. Because of the specificity of the GSA-AT enzyme and the plant origin of the marker, we think that it could be considered a favourable alternative to currently used SMGs both for the risk assessment process and for public acceptance.

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Poster Abstract – 7.51

CHARACTERIZATION OF T-DNA INTEGRATION EVENTS IN ALFALFA

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Agrobacterium tumefaciens, flanking sequences, genetic transformation, Medicago sativa, vector backbone

Integration of foreign DNA into the plant genome is a natural phenomenon mediated by the bacterium *Agrobacterium tumefaciens*. The "transformation machinery" of *Agrobacterium tumefaciens* has been studied extensively at the level of T strand production and integration. T strand production, either from natural or artificial plasmids, shows high variability, mostly due to inefficient termination at the left border (LB) during T-strand generation, and many studies have revealed integration of vector backbone sequences (VBS) in the plant genome along with the desired genes.

A great contribution to the current knowledge comes from the studies conducted on the model species *Arabidopsis thaliana* and *Oryza sativa* but no data are available for other crop species, including *Medicago sativa*. For this reason the characterization of the integrations events in several transgenic plants of alfalfa, available in our laboratory, was undertaken.

We designed a multiple PCR assay in order to evaluate the frequency of vector backbone integration in T_0 transgenic plants.

The percentage of events containing VBS ranged from 25 % to 33 % and it was consistent with the results reported in the literature for other species. Transmission to the progeny of the VBS was also demonstrated in T_1 plants.

In order to show the rearrangement occurred at the LB and RB the junction sequences between T-DNA and the plant genome were isolated and amplified by inverse PCR (iPCR). The amplicons produced are being sequenced. Southern blot analyses will also performed to confirm the PCR-based evidence of VBS integration. The implications of these results in the development of transgenic plants of alfalfa are discussed.

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Poster Abstract – 7.52

PHYLOGENETIC RELATIONSHIPS IN THE GENUS *NICOTIANA* ASSESSED BY MEANS OF AFLPs

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Nicotiana, AFLP, phylogeny, origin cultivated tobacco

Phylogenetic relationships in the genus *Nicotiana* were investigated using AFLP analysis and the results were used to evaluate previous hypotheses about the origins of these taxa. Phylogenetic analysis of AFLP data were performed on the entire genus (60 of the 77 naturally occurring species, plus some artificial interspecific hybrids), comprising both diploid and polyploidy taxa to examine the effects of amphidiploids on estimates of relationships. Results are compared with published phylogeny using fewer, but many of the same, taxa. The patterns of relationships in *Nicotiana* are largely congruent with previous evolutionary ideas based on morphology and cytology with few important differences. The occurrence of *Nicotiana tabacum*, the commercial tobacco plant, in the wild state and its origin and evolution are of great interest especially now that *N. tabacum* is proposed as an energy crop and the evolutionary direction has been reversed. *N. tabacum* (*n*=24) is believed to have arisen by chromosome doubling after hybridization of *N. sylvestris* (*n*=12) with a species in the Tomentosae section of Nicotiana. Goodspeed and Clausen suggested that *N. tomentosa* Ruiz and Pavon (n=12) was the species but Clausen changed it to *N. tomentosiforms* Goodspeed (*n*=12). Goodspeed changed to *N. otophora* Grisebach (*n*=12). Later evidence, however, suggest *N. tomentosiformis* as the more likely progenitor of *N. tabacum*. Gerstel, from an analysis of the segregation of artificial polyploids of *N. tabacum x N. tomentosiformis* and *N. tabacum x N. otophora*, concluded that *N. tomentosiformis* showed the greater chromosome homology with *N. tabacum*.

Goodspeed *Nicotiana* studies based on morphology are congruent with recent molecular analysis (Chase et al. 2003) and a reclassification of *Nicotiana* is not yet provided because there is evidence that the evolution of the genus is more complicated than has been thought previously.

Our analysis of diploid and polyploid species with inclusion of different amphidiploid species and interspecific hybrids may be able to detect the products of recent hybridization events responsible of the evolutionary pattern.

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Poster Abstract – 7.53

TIPIPAPA: A NATIONAL PROJECT TO CHARACTERIZE AND TRACE EARLY POTATO PRODUCTION

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Solanum tuberosum, molecular fingerprinting, secondary metabolites, mineral and multielement analysis, cost-benefit analysis

TIPIPAPA (Tipicizzazione e caratterizzazione di varietà precoci di patata con l'impiego di tecniche molecolari e spettroscopiche) is a national research project funded by MiPAF. It was conceived with the idea of promoting research activities and developments related to the traceability, characterization and valorization of potatoes produced in southern Italy. A research main target is the genomic, proteomic, transcriptomic and metabolomic characterization of potato varieties normally grown off-season in southern Italy. In these areas early potatoes represent an essential element in the exportation of agricultural products. In addition, the mineral, multi-element and Sr-isotope ratio analyses on soil and potato samples are carried out to find soil-related indicators of potato's geographical origin. Potatoes are sampled in Campania, Apulia and Sicily regions each year, and lyophilized samples are distributed to the six research units for the analyses. Together with traceability and characterization, TIPIPAPA also includes the phytopathological screening of potato in the production areas, the definition of protocols ascertaining the presence of transgenes in tubers, and a program that evaluates economic aspects and costs-benefits related to the potato traceability pipeline For more information www.pbglab.com.

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Poster Abstract – 7.54

METABOLITE PROFILES AND MORPHO-ANATOMICAL TRAITS IN LEAVES OF POTATO ORYZALIN INDUCED POLYPLOIDS

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polyploids, Solanum, leaf area, stomata parameters, secondary metabolites

In view of the widespread success of polyploid plants, it appears that polyploidy confers adaptive advantages. It is well known the relationship between ploidy and body/organ sizes (tetraploid plants are larger than their diploid parents in terms of leaf, flower, and cell size in the leaf lamina) as well as the effects of chromosome doubling on whole genome expression (changes in regulatory networks and output pathways are usually observed in polyploids). However the mechanisms underlying such phenomena are still poorly understood. Potato provides an excellent model system for studies on the consequences of polyploidization, since the genus *Solanum* shows an exceptional tolerance of ploidy manipulation. The aims of this study were to produce 4x genotypes from two 2x wild species (*S. commersonii* and *S. bulbocastanum*) and to characterize obtained polyploids from the morpho-anatomical and biochemical standpoint. Nine 4x genotypes (five from *S. commersonii* and four from *S. bulbocastanum*,) were produced by oryzalin treatment and analyzed. While in *S. commersonii* the leaf area reduced with increased ploidy level, in *S. bulbocastanum* two 4x genotypes showed a leaf area larger than the 2x progenitor and two did not. Results from analysis of stomata frequency (per mm^2) and length (guard-cell length, pole to pole) showed significant differences between 2x progenitors and 4x genotypes. However, we did not observe a clear relationship between stomata parameters and polyploidization. As for metabolite profiling, the analysis of 4x genotypes in *S. commersonii* showed that leaf content of total alkaloids was significantly lower in the 4x genotypes than in the 2x progenitor. By contrast, 4x genotypes showed an increased amount of some phenol compounds, as caffeic and ferulic acid and rutina. To elucidate the molecular events associated to polyploidization, genome expression and methylation analysis are being carried out. Using the whole transcriptome approach screening of microarray, we aim to get a snapshot on gene expression modulation after polyploidization. Moreover, citosyne-directed methylation patterns will be studied using MSAP (Methylation Sensitive Amplified Polymorphism) approach.

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Poster Abstract – 7.55

ASSESSMENT OF *IN VITRO* PARAMETERS SUITABLE FOR EARLY IDENTIFICATION AND SELECTION OF TOMATO PARTHENOCARPIC MUTANTS

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parthenocarpic mutants, in vitro culture, Solanum lycopersicum, GA_3, NAA

Unfavourable conditions, such as relatively low temperatures, short photoperiod, high umidity can reduce pollen production, anther dehiscence and, as a consequence, fruit set. The parthenocarpic growth of the ovary into a seedless fruit without pollination and/or fertilization is a very attractive trait for the breeders and has been extensively studied in tomato, where natural, facultative parthenocarpy sources are known (*pat*, *pat-2* and *pat-3/pat-4*).

The *pat* gene, obtained following EMS seed treatment, is recessive and has pleiotropic effects, conferring a phenotype with short anthers, fruits smaller than the normal and with a reduced number of seeds, also in favourable environmental conditions. Developmental studies showed that the growth of the ovary in the *pat* mutant, unlike the normal ones, begins at the pre-anthesis stage.

Natural mutants for parthenocarpy better express their phenotype in presence of long photoperiod and low night temperatures. During autumn and winter season or when the environmental conditions are favourable to normal fruit setting, the identification of these mutations is challenging because of low penetrance and/or expressivity.

The early identification and the selection of tomato parthenocarpic mutants when the environmental conditions hamper their expression could be facilitated by the availability of simple and reliable screening methods. To this purpose, the *in vitro* sensitivity of normal (WT) and *pat* vegetative and reproductive organs were studied in near isogenic lines.

Different concentrations of auxin (NAA) and gibberellic acid (GA_3), alone or in combination with the respective inhibitors 2,3,5-Triiodobenzoic acid (TIBA) or Paclobutrazol (PAC) were used. The doses were assessed on plantlets derived from germinating seed or on flowers harvested at pre-anthesis stages. Main root length, number of secondary roots, hypocotyls height, weight, radial and polar diameter of the ovary were recorded.

Due to its unbalanced hormonal content, the behaviour of *pat* mutant was clearly distinguishable from the corresponding WT. On the plantlets, both auxin and GA_3 increased the number of secondary lateral roots in the parthenocarpic line, while both hormones favour the development of the aerial part in the WT version.

After twenty days of *in vitro* culture, the presence of GA_3 in the medium determined the swelling of ovaries in both WT and *pat* versions in comparison to ovaries grown on media without hormones; a greater development of *pat* ovaries was observed when organs were dissected at an earlier floral stage. The NAA determined an enlargement only in the *pat* ovary, suggesting a particular role integrating that of GA_3. Finally, the action of both hormones used did not restore a normal anthers development in the *pat* line.

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Poster Abstract – 7.56

GENETIC VARIABILITY OF POPULATIONS OF GIANT REED (*ARUNDO DONAX* L.) ASSESSED BY MEANS OF AFLPs AND RAPDs MOLECULAR MARKERS

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biomass, energy production, Arundo donax, genetic variability, molecular markers

Cultivations of biomass for energy production cover a strategic role in the near future of European agriculture. Among these perennial crops, because of high yield potentials and collateral environmental benefits, have a major importance. Giant reed (*Arundo donax* L.) is an herbaceous perennial that has attracted a lot of interest both for its very high potential yield and for the adaptability to a large range of environments. In particular *A. donax* has been considered one of the most promising species for energy production in Southern Europe.

Actually a very limited knowledge concerning both the genetic variability of different *A. donax* populations and how the genetic variability could influence plant production is available, and this could represent a limiting factor to a large diffusion of *A. donax* cultivation.

In the present work the genetic variability of several accession of A. donax from different Italian and European origin was evaluated by means of fluorescent AFLP (Amplified Fragment Length Polymorphism) and fluorescent RAPD (Random Amplified Polymorphic DNA) markers.

These two class of markers were selected because of their great flexibility with respect to other classes of markers making them very useful for the analysis of species like *A. donax* characterised by few molecular data.

Preliminary results obtained using a single AFLP primer combination and five different RAPD markers showed that the genetic variability was very low independently from the site of origin of the sample (Italy, France, Spain, and Greece) presenting generally a single multilocus fingerprint. This situation is compatible, excluding few simple mutations, with the presence of a single genetic clone spread in the Mediterranean area.

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Poster Abstract – 7.57

SUNFLOWER AS A WINTER CROP IN MEDITERRANEAN AREA

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sunflower breeding, low input cultivation, Mediterranean environments

Water deficit represents a limit for sunflower (*Helianthus annuus*) in Mediterranean environments. It is possible to consider winter sowing as strategy to let sunflower to overlap rainy seasons during the delicate phases of vegetative growth, thus optimising the exploitation of soil water content due to seasonal rainfall conditions.

The goal of this experiment was to investigate the performances of some *H. annuus* hybrids winter sowed without water supply in a Mediterranean clime and to get new information on qualitative and quantitative traits.

Eleven sunflower hybrids were evaluated at two locations in Southern Italy, in three winter sowing dates for two years. The experimental trial adopted was a complete block design with three replications. Any water regimen was supplied and water rainfall and daily temperatures were recorded. Furthermore, as a control, the hybrids underwent to conventional sowing date and standard water regimen.

The most important agronomic traits (days to emergency, plant height, areic yield, oil content) were recorded and statistically computed with ANOVA procedure. Mean values were computed with SNK test and a Multiple Regression Analysis was calculated.

Significant differences were scored for all the hybrids and traits recorded in the two locations, and between winter and conventional sowing. In general, a negative correlation between some yield components and oil content was observed in both environments.

The obtained results showed as expected a better response of hybrids under conventional field conditions with respect to winter sowing practice. Anyway, oil content and yield components recorded in unconventional cycle without any water supply, could led to consider sunflower as a possible alternative crop to wheat monoculture in Southern Italy. Further investigations are in progress to select new hybrids by breeding programmes.

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Poster Abstract – 7.58

SunTILL: A SUNFLOWER TILLING PLATFORM FOR FUNCTIONAL ANALYSIS OF GENES INVOLVED IN FATTY ACID BIOSYNTHESIS

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sunflower, TILLING population, fatty acids

Plant oils represent renewable natural resources, useful in several economic sectors, from food to specialty chemicals and high added value products. Most marketed plant oils contain the most common C:18 fatty acids, i.e. stearic, oleic, linoleic and linolenic acids. Sunflower oil is considered a polyunsaturated oil for its high linoleic acid and low saturated fatty acid contents. The highly polyunsaturated nature of this oil makes it an interesting multi-purposes product. Until now, sunflower breeding programs aimed to the identification of genetic variants have been carried out and improved lines for stearic and oleic contents have been selected. Anyway, the narrow genetic variability, as a consequence of selection programs for high oil yield developed in the last century, suggests the need of innovative techniques to obtain new genotypes with a modified lipidic composition. To this aim, TILLING (Targeting Induced Local Lesions IN Genomes) represents a powerful tool to identify novel genetic variation in genes that affect key traits.

A kill-curve analysis was carried out on 8 seeds batches in order to discover the optimal experimental conditions (different EMS concentrations and exposure times). According to the germination rate, a treatment with 0.7% EMS for 6 hours was chosen and applied to the whole M0 population. Thereby, a stock of 30.000 seeds was mutagenized. To avoid ambiguities caused by the chimerism of M1 generation, the obtained 4211 plants were self-fertilized; finally, an M2 population of 3553 plants was developed and used to create the DNA TILLING library. Obtained plants were previously analysed for the main phenotypic characters, following the guidelines arranged by IPGRI (International Plant Genetic Resource Institute, Italy), and a photographic catalogue was created. From each plant, three adult leaves were collected, dried, stored and used for subsequent DNA extraction. Since this step represents a critical point of the TILLING technique, different extraction methods were tested. Moreover, a microsatellite analysis was carried out in order to evaluate the presence of any Taq polymerase inhibitors. To set up the TILLING technique on sunflower genome and to reduce the background from LiCOR gel imagines, a preliminary CelI-nuclease mismatch cleavage assay, with different enzyme concentrations (1:2, 1:4, 1:10, 1:20 dilutions) and different digestion times (15-30-45 minutes), has been performed. The best CelI activity resulted for the highest dilution and the longest incubation time, so these conditions will be applied for the screening of the whole population. Expressed Sequences TAGs (ESTs) of genes involved in fatty acids biosynthesis (Acetyl CoA carboxilase, 3-chetoacyl-ACP synthase type II and III and so on) have been website-selected and analysed by bioinformatic tools, in order to

reconstruct the gene models and to identify the genomic regions most suitable for TILLING screening. PCR conditions for each primer pairs were optimized to obtain an extremely specific product with optimal length for TILLING analysis (700-1500 bp).

Thereby, based on the production of a mutagenized population and on an high throughput identification of EMS-induced point mutations in specific target genes, TILLING represents a powerful tool to produce novel genetic variation and to study the gene function in genotypes of agronomical importance, without the creation of transgenic material.

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Poster Abstract – 7.59

GENETIC DIVERSITY AND REPRODUCTIVE BIOLOGY OF *JATROPHA CURCAS* L.

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population genetics, apomixis, molecular markers, flow cytometric seed screens, biodiesel

Jatropha curcas L. is a drought-resistant, photo-insensitive perennial tree plant belonging to the spurge family (*Euphorbiaceae*). This species probably originated in Mexico or neighbouring parts of Central America, but it was introduced all over the world long ago and is now naturalized throughout the tropical and subtropical areas. *J. curcas* is becoming an increasingly popular oleaginous crop in several developed countries for its proposed value in the lipids, biopharmaceuticals, cosmetics and biopesticides industry. Mainly it is known as a source of oil-rich seeds (*i.e*. fat content of whole seeds varies up to 45%) traditionally used for the production of soap and biofuel. It is worth mentioning that the seed oil is not edible as it contains toxic compounds and antinutritional factors. Despite the potentials as a source of vegetable oil for the replacement of petroleum and the interest that is being shown in the large-scale plantation systems of *J. curcas* in newly cultivated areas of Africa, America and Asia, the genetic structure of local varieties remains poorly characterized and breeding programs for the selection of improved varieties are scanty in this species.

J. curcas is a monoic plant with unisexual flowers, being male and female flowers produced in the same raceme. On the basis of available information, 68% of seeds are set through amphimixis, mainly by outcrossing (enthomophylous pollination), even if the species is self-compatible and hence selfing is also possible. At the population level, the average degree of apomixis is equal to 32%. Agamospermy (*i.e*. embryo sacs and embryos produced in ovules without meiotic reduction or egg cell fertilization), as a mode of asexual reproduction through seed, leads to clonality. Nevertheless, the species seems also to show a tendency to promote xenogamy (*i.e*. union of genetically unrelated organisms) and to minimize geitonogamy (*i.e*. the pollination of a flower with the pollen from another flower on the same plant), mechanisms that increase diversity.

The aim of this work is to gain an insight into the population genetics and reproduction dynamics of *J. curcas*. This species is characterized by a relatively small genome size (2*C*=0.85), corresponding to about 430 Mbp: it has a basic chromosome number equal to 11, and its populations are composed mainly of diploids (2*n*=2*x*=22), although triploid and tetraploid chromosome numbers have rarely been reported. The determination of ploidy and the reconstruction of reproductive strategy in local populations of *J. curcas* from India, Sry Lanka, Brazil, Peru, Mexico, Nicaragua, Somalia, Togo, etc. were determined by means of flow cytometric seed screen (FCSS), a method suitable for the discrimination of either pseudogamous or autonomous apomixis from sexuality (*i.e*. amphimixis) based on the seed DNA contents of embryo and endosperm. The investigation of genetic variation within and differentiation among populations was carried out exploiting nuclear DNA markers, mainly AFLP (amplified fragment length

polymorphism) and SSR (simple sequence repeat) markers, whereas phylogenetic relationships were reconstructed on the basis of SNP (single nucleotide polymorphism) markers for chloroplastic DNA genic and intergenic regions.

A more profound knowledge of the mechanisms that regulate reproductive events and that affect the genetic structure of populations in *J. curcas* would contribute fundamentally to understanding i) the patterns of seed formation, ii) the potentials of genetic recombination, iii) the dynamics of natural populations, and iv) the strategies for breeding improved varieties.

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Poster Abstract – 7.60

DEVELOPMENT OF NOVEL SSR MARKERS FROM A GENOMIC MICROSATELLITE LIBRARY IN *JATROPHA CURCAS* L.

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Jatropha curcas L., energy crop, microsatellite, genomic library, polymorphism

Jatropha curcas is a drought-resistant shrub belonging to the family Euphorbiaceae. It is native of Central America and traditionally cultivated as living fence and for traditional use in many tropical and sub-tropical countries.

This specie is easy to establish, it can be grown in degraded land and in low rainfall adapted to high level of aridity and to soils with low nutrient content.

Jatropha is a genus with a multipurpose uses, like medicinal, fertilizers, soap, biomass production and currently, there is a great interest through a high potential as a energy crop. A plantation of *J. curcas* can produce high yields for many years and the seeds contain an average of 35-40% of not-edible oil that can be employed as pure oil or for bio-diesel production. This plant does not compete with land resources for food and feed.

At the moment limited is the genetic information of this species and few studies have focussed on the germplasm characterisation using molecular markers.

The objective of this research was to develop a genomic library enriched for microsatellites in order to generate a large number of SSR markers suitable for evaluating genetic polymorphisms in *Jatropha curcas* accessions.

A genomic library, enriched for di- (GA, GT), tri- (CAA, ATT) e tetranucleotides (GATA, CATA) using the method of Edwards *et al*. (1996), has been constructed.

From the library, 226 sequences were obtained, of which 70 (30%) displayed microsatellite sequences. Using the program entitled PRIMER 3 (Rozen & Skaletsky 2000), 20 pairs primers were designed and have been tested.

The development of a genomic library enriched in microsatellites, could be useful to generate an adequate number of SSR markers sufficiently polymorphic to implement population studies and useful for the set up of future programs of genetic improvement.

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Poster Abstract – 7.61

INTRONS ARE IMPORTANT PLAYERS OF TUBULIN GENE EXPRESSION: WHAT'S THE ROLES?

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intron, gene expression, IME, transient transformation

The control of gene expression in eukariotes is the result of a complex network of pathways acting at multiple steps from transcription to translation. This control is exerted by a strict cooperation between proteins and nucleic acids.

Among the most recent actors in this scenery are introns, whose regulatory role have emerged in higher eukariotes. IME (Intron mediated enhancement of gene expression) is the best known regulatory effect exerted by introns within a transcription unit. In rice plants, we have shown that introns of tubulin genes can sustain IME and, in addition, can also influence the site of gene expression, determining tissue-specificity: this should be considered when testing new promoters for transgene expression. Introns may contribute to the evolution of those gene families where coding sequences are subjected to strong functional constraints. In fact, intron sequences, that are left to evolve more freely, can contribute to new pattern of expression. Several examples of enhancing introns have been reported in plants, but their mode of action is still elusive.

A series of recombinant plasmids, based on the regulatory sequence (promoter-leader-intron) of rice tubulin genes, have been used to get new insights on intron-mediated control of gene expression, through transient expression assays and transgenic plant production. As a result, we show that introns act post-transcriptionally, that splicing is fundamental for intron function and that different and specific determinants act in monocot and dicot species. Interestingly, low reporter gene expression from a rice promoter in Arabidopsis or tobacco cells, is not enhanced by the corresponding intron but can be rescued by the addition of a dicot intron.

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Poster Abstract – 7.62

IDENTIFICATION OF NEW TRANSCRIPTION FACTOR BINDING SITES INVOLVED IN TRANSCRIPTIONAL COORDINATION OF THE OXIDATIVE PHOSPHORYLATION GENES IN THE *A. THALIANA*

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oxidative phosphorylation gene, mitochondrial, Arabidopsis thaliana, transcriptional coordination

The energy state of the cell is the bottleneck for the vast majority of metabolic processes. The identification of genetic determinants involved in regulating energy metabolism may reveal the connections between energy metabolism and other biological processes.

In *Arabidopsis thaliana*, different studies provided evidence for the co-regulation of the genes involved in mitochondrial pathways. However, the molecular dissection of the mitochondrial regulatory network is still in its early infancy.

Mitochondrial biogenesis requires the expression of two separate genomes. About 5% of mitochondrial proteins are encoded within the organelle, while the remaining 95% are encoded in the nuclear genome (Adams and Palmer, 2003; Mackenzie and McIntosh, 1999). In all eukaryotes, most of the proteins encoded in the mitochondrial genome are components of the oxidative phosphorylation (OXPHOS) machinery. The formation of multi-subunit complexes requires a stechiometric assembly of their components. Even if the coordination of the expression of nuclear genes occurs at different levels, the transcriptional regulation is the prominent. The interaction of common sets of transcription factors with cognate binding sites is present in the canonic regions of regulation (Welchen and Gonzalez, 2006).

We decided to investigate the OXPHOS nuclear genes in *Arabidopsis thaliana* in order to identify new transcription factor binding sites in canonic (promoter) and non canonic (introns and UTRs) regions of regulation. The set of OXPHOS nuclear genes, obtained from *Arabidopsis thaliana* genome database *(TAIR)* have been analyzed by different matrix-based pattern discovery bioinformatics tools, such as *Consensus, MEME and Bioprospector*, to extract shared motifs from the OXPHOS set of unaligned sequences. We tested the *genome-wide* distribution and the significance of these motifs by performing pattern-search term analysis. The results show that a set of 5 motifs is present in the whole respiratory chain gene set, whereas the same motifs are present in a lower percentage in the remaining genes of *A. thaliana*.

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Poster Abstract – 7.63

CHARACTERIZATION OF ORNAMENTAL PLANTS OBTAINED BY INTERSPECIFIC HYBRIDS OF *NICOTIANA* SPECIES

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Nicotiana spp, ornamental plants

A programme of genetic improvement has been carried out to select intraspecific hybrids of *Nicotiana* spp. with characteristics of ornamental plants. In the germplasm collection of CRA-CAT Research Unit *Nicotiana* species having several characters of floricultural interest are present. For this research the following characters have been considered: the size of plant, the erect look, the dimension, colour and fragrance of flower, and the persistence of flowering. The species utilized to obtain interspecific hybrids F1 were *N. sylvestris* (2n=18); *N. alata* (2n=18); *N. forgetiana* (2n=18); *N. suaveolens* (2n=32) e *N. sanderae* (2n=18). Morphological investigation and cytological analysis carried out on the number of chloroplast present in the cell guard of the stoma as well as on the number of chromosomes determined in the cells of root apex, revealed relevant differences among the F1 hybrids and the parental species. Fertile F1 hybrids, selected for ornamental characteristics, were submitted to self-pollination, backcross and androgenesis.

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Poster Abstract – 7.64

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION METHOD FOR DETECTION OF *TRANZSCHELIA DISCOLOR* INFECTION IN *ANEMONE CORONARIA* TISSUES

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loop-mediated isothermal amplification, DNA extraction, rust, Anemone coronaria, Tranzschelia discolor

INTRODUCTION. The *Prunus*/*Anemone* rust is caused by the fungus *Tranzschelia discolor*, that has become aggressive in *Anemone* cultivation in recent years. During the growth cycle required to produce rhizomes from seeds, *Anemone* seedling became infected by basidiospores derived from teleutospores formed on *Prunus* leaf; infected plants remain asymptomatic. The disease appears in the next vegetative cycle, on plants cultivated to crop flowers. *T. discolor* infection dramatically reduces flower production and quality. Ecidiospores released from *Anemone* leaves only infect *Prunus*. The ability to detect for the pathogen in infected tissue represents the best, environmentally friendly, means for disease control in cultivations. Here we present the application of loop-mediated isothermal amplification (LAMP) for the detection of *T. discolor* and show that this method has many advantages when compared to the classical PCR amplification method.

MATERIALS. *A. coronaria* fresh leaves and dried rhizomes were used in the experiments. Fresh tissue were employed directly or frozen and stored at –80°C and subsequently utilized for DNA extraction.

METHODS. The design of the oligonucleotides was based on the *T. discolor* gene for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequences (GenBank: AB097449.1). A commercial DNA extraction kit was used to prepare the genomic DNA. LAMP and PCR were carried out in a Tgradient thermal cycler (Biometra) using appropriate conditions. PCR amplification products were recovered from gels for sequencing.

In addition, for LAMP analysis, rhizome sections were disrupted in a buffered solution and directly used as template for amplification in a thermal block. LAMP products were analyzed by either ethidium bromide staining on agarose gels or directly detected in test tubes, using hydroxy naphtol blue or calcein.

Dried rhizome sections were coloured using Trypan Blue assay, a methodology for mycelium identification, and analysed using microscopy (model Leica DM4000b) in parallel to the molecular analysis methods.

RESULTS AND DISCUSSION. Both PCR and LAMP methods presented here permits to identify the infection in apparently healthy rhizome, but LAMP allows the detection of the pathogen directly from tissue without the need for DNA extraction. Moreover, it does not require the use of

laboratory equipment (e.g. thermal cycler, UV trans-illuminator, power supply, gel electrophoresis chamber) as the method is isothermal and detection can be done directly.

The LAMP methodology could also be used in breeding project as a simple methodology for preliminary screening of *T. discolor* resistant progeny.

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Poster Abstract – 7.65

INTERSPECIFIC HYBRIDISATION IN *ANEMONE* SPP.

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genetic resources, hybrid fertility, plant breeding

The genus *Anemone* (*Ranunculaceae*) includes about 85 species. The Mediterranean basin is the centre of differentiation for many of the species that have contributed to presently cultivated varieties: *A. coronaria*, *A. hortensis*, *A. pavonina* and *A.* x *fulgens*. Wild populations of *A. coronaria*, the most valuable species, are frequently found in northern Mediterranean littoral, from France to Italy, Greece, Southern Turkey, up to Northern Iraq, Syria, Israel and Northern Africa (Horovitz et al. 1975. Journal of Botany 24, 26–41). *A. coronaria* is a winter-flowering species, (Yonash et al. 2004. Euphytica 136, 51–62.) and the progenitor of the majority of varieties currently grown for cut-flower production and garden plants. *A. hortensis*, *A. pavonina* and *A.* x *fulgens* (likely an interspecific hybrid between *A. hortensis* and *A. pavonina*), are also of mediterranean origin, and grown mostly as garden plants. These three species cluster together on the basis of ribosomal and chloroplast DNA restriction sites variation and are closely related to *A. coronaria* (Hoot et al., 1994. Systematic Botany 19: 169-200). All the other *Anemone* species were less closely related to *A. coronaria*. Maya and Venard (1976), reported by Maynet (1993. In: The physiology of flower bulbs 211–218), found that the *A. hortensis* x *A. pavonina* hybrid was fertile but the *A. pavonina* x *A. coronaria* hybrid was sterile due to failure of chromosome pairing. Notwithstanding this preliminary disappointing knowledge, interspecific crosses were attempted between the four *Anemone* species aimed to transfer useful traits and to create new genetic variations. In fact *A. coronaria* carry a wide variation in flower colours, large flower on robust stalk. The related specie show foliage traits suitable for pot plant production and a yellow/cream colour laking in *A. coronaria*. The following crosses were made: *A.c.* (2n) x *A.h.*; *A .c.* (2n) x *A.f.*; *A.c.*(4n) x *A.f.*; *A.c.*(4n) x *A.p.*; (*A.f.* x *A.p.*) x *A.c* (2n); (*A.f.* x *A.p.*) x *A.f.*; *A.p.* x *A. h.*; *A.f.* x *A.p.* (F2). Plants derived from one-year-old rhizomes were evaluated to confirm their origin, to verify their fertility and to check aesthetic and agronomic performance. Plants of the hybrids involving *Anemone coronaria* were female and male sterile as demonstrated by selfing and backcrosses. Most of the hybrids showed intermediate traits or traits of both parents. Scheduled attempts to rescue fertility include chromosome doubling by chemical treatments or anther cultures. Crosses concerning *A. pavonina, A. hortensis* and *A. x fulgens* were fully fertile. *A.p.* x *A.h.* crosses showed vigorous plants with flowers of a predominant lavender colour appropriate for creation of spots in large gardens. The progeny derived by open pollination from the cross *A.f.* x *A.p* carries a set of characters of the flowers (colours ranging from white to yellow/cream, pink, lavender, red and cyanic) and of the foliage (short petiole, scarcely lobed and erect leaves) that make them suitable for pot plant production. These materials may convey into ultimate breeding steps finalised to produce *Anemone* pot cvs; they attracted so far the interest of breeder companies.

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Poster Abstract – 7.66

MOLECULAR APPROACHES. A CONTRIBUTION TO THE CLASSIFICATION OF THE GENUS *RHODODENDRON*

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AFLP, STMS, EST, old hybrids, azaleas conservation

Rhododendrons and azalea are the most popular flowering, broadleaf evergreens and landscape plants in the Northern Hemisphere. The cultivated types are hybrids obtained by means of complicated cross-combinations of many species, mainly with Asian origin. The first recorded cross was made in 1800 between *R. ponticum* and *R. nudiflorum*. During the end of the 18th century the hybridisation activity reached remarkable levels. As for most of the cultivated tree species, the identification of rhododendrons is convoluted and the same cultivar name has been accidentally given to more than one genotype. Conversely, some cultivars have several synonymous names.

A better understanding of the effectiveness of the different DNA-based markers is an important step towards plant germplasm characterization and classification. Comparisons of the performance of several molecular markers in assessing genetic diversity have demonstrated that correlations between the genetic relationships revealed by different polymorphism techniques can vary widely. For this reason AFLP, STMS and EST markers were compared to determine their relative efficiencies in a study of genetic diversity among 84 evergreen azalea, an important source of germplasm almost unknown. Three AFLP, 6 STMSs and 9 EST primer pairs were used. Statistical analyses were carried out in order both to compare markers and to establish horticultural classification. High level of polymorphism was observed for all three marker systems, but AFLPs appeared to be the most efficient marker system due to their highest polymorphism detection capacity. Similarity matrices produced for each marker technique showed weak, yet significant, correlations (statistic *g*) when Mantel test was applied. The highest correlations were observed between AFLP and STMS. The Analysis of Molecular Variance showed that for all markers the genetic diversity was mainly attributable to differences among cultivars within horticultural groups. By means of assignment test and cluster analyses cultivars were grouped with a remarkable efficiency in their source population. In conclusion, the selected AFLPs thanks to their high polymorphism detection capacity and genome coverage resulted to be the most appropriate markers for studying the origin of azalea hybrids. On the other hand, STMS and EST markers appeared to be the most appropriate markers for paternity assessment of narrow genetic relationships.

Then, in order to characterized and classified an important rhododendron collection, 4 STMSs, developed in *R. catawbiense* were applied to 33 accessions collected in the Burcina Park (Northern Italy). Fingerprinting revealed a total of 79 alleles with an average of 19.75 alleles per locus. PCo analysis was firstly performed only on 17 morphological traits but could not differentiate hybrids. Indeed, no significant correlation between molecular and morphological data was detected. Probably caused by the STMSs, considered as neutral markers, while morphological traits are

subject to environmental factors. However, combining STMS and morphological data sets allowed to obtain a more comprehensive representation of the genetic relationships among plants. In particular, some hybrids with unknown parents were grouped closed to *R. catawbiense* such as 'Memoire de Dominique Vervaene'. Thus STMss appeared to give useful information for horticultural classification.

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Poster Abstract – 7.67

STMSs MARKERS DEMONSTRATE GENETIC DIFFERENTIATION IN ORNAMENTALS GENUS: THE CASES OF OLD GARDEN ROSES AND CAMELLIAS

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microsatellites, Rosa, Camellia spp., classification, biodiversity

Genetic resources can be described as the total genetic diversity of cultivated species and their wild relatives. Effective conservation and use of plant genetic germplasm depend on the availability of information and on the extent and distribution of genetic diversity in species of interest. Such information allowed to improve conservation management and help users to find material with desirable characteristics.

In recent years, numerous molecular techniques have been developed to provide markers and now are by far the most powerful and widely used. Among the several classes of DNA-based markers, the STMSs (*Sequence Tagged Microsatellite Sites*) are highly polymorphic, multi-allelic, frequently co-dominant, highly reproducible, selective neutrality and random widely distributed in the genome. In order to improve the genetic knowledge in ornamentals and to solve taxonomical issues, STMSs were applied in two among the most economically important plants: garden roses and camellias.

Roses have been cultivated as ornamental plants for more than 2000 years. The existence of many species together with the high level of interspecific and inter-sectional hybridisation make confused the genetic relationships within the genus. A phylogenetic analysis on 18 species and 47 cultivars using STMSs markers was carried out to clarify the classification of Old Garden Roses (OGRs) and quantify the discriminating power of the loci. Six microsatellites primer pairs, located on at least 4 different linkage groups, were selected. A total of 82 alleles were detected with an average of 13.7 alleles per locus. The number of allelic phenotypes per locus ranged from 15 to 50 with an average of 34.7. The UPGMA clustering of Jaccard similarities was in good agreement with the botanical classification and with previous phylogenetic approaches based on *mat*K and on cpDNA analyses, confirming the relative position in the dendrogram.

Taxonomical problems mainly due to natural inter specific hybridization occur also in the genus *Camellia*. Many species are economically important plants with ornamental value, such as *C. japonica* and *C. sasanqua*. A total of 132 accessions belonging to *Camellia* spp., including *C. sasanqua*, *C. japonica*, *C.* x *vernalis*, *C.* x *hiemalis* and *C. hybrida* cultivars were analyzed by 4 STMSs developed in *C. japonica*. These markers successfully amplified 24 species, demonstrating their cross-transferability. The constructed PCoA (Principal Coordinate Analyses) showed a distribution of all accessions in agreement with their taxonomic classification. The spatial

distribution on the scatter plot, allowed to demonstrate that the STMSs were able to describe genetic diversity both in winter and spring camellias.

In conclusion, the good STMS cross-species transferability observed in roses and camellias makes these sets of microsatellite loci an appropriate tool for germplasm characterization and management.

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Poster Abstract – 7.68

HYBRID-TEA ROSE BREEDING: APPROACHES TO INCREASE SEED PRODUCTION AND GERMINATION

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fertility, GA3, mean germination time, pre-germination, Rosa x hybrida L.

Hybrid-Tea rose breeding has worldwide great economical importance for production of new cultivars of roses (*Rosa x hybrida* L.). Seed production and germination are important bottlenecks for breeding companies which press for novelty by increasing phenotypic variability. Moreover, the production of germinating seeds is generally lower than many other crops, often not higher than 30%, with hip content usually ranging between one to 30 seeds. The selection of fertile parents, by detection of fertility related markers, could be a strategy to raise seed production and to obtain viable and germinating seeds. Indeed, environment interactions during seed stratification, combining the action of cell-wall macerating enzymes with hormone pre-germination treatments, could also optimize some aspects of the germination process.

In 2008 a high and a low fertile pollen donor and a high and a low fertile seed parent, selected from a company breeding database, were crossed in partial diallel. More than 600 hybridizations produced a total of 22011,00 seeds, with a value of 60,2 (seeds/hybridization) for the crosses between the two fertile parents, a value of 0,04 for the crosses between the two low fertile parents, a value of 9 for the crosses between the low fertile seed parent and the high fertile pollen donor and a value of 1,78 between the fertile seed parent and the low fertile pollen donor. The recovered seeds were cold stratified at 4°C in sand for 40 days, interlaced by warm treatment at 20 °C for 22 days. Pre-stratification treatments were applied in combination or not with seed sterilization, compost activator, artificial inoculations with either a mix of fungi or a mix of bacteria or *Alternaria* sp. (previously isolated from of the rose hips) or a treatment with *Trichoderma* sp. (commercial formulation). Pre-germination treatments were also applied by soaking the stratified seeds overnight in solutions containing the macerating enzyme driselase and the hormone GA_3, at different concentrations and in combination between them. Seeds were finally sown on cold perlite beds at a germination density of 209 seeds/m^2, in the greenhouse. The mean germination time and the percentage of seed germination were evaluated for each treatment. Parents fertility and environmental factors affect rose seed production and germination, therefore they should both be considered in a successful breeding strategy. In order to increase the possibility of obtaining new cultivars, further studies on fungal contamination, seed vitality and physiological or physical dormancy will be carried out. Besides, morphological, genetic and molecular approaches are currently attempted to identify possible markers related to fertility.

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Poster Abstract – 7.69

ANALYSIS OF THE *VHB* HAEMOGLOBIN GENE FROM *VITREOSCILLA STERCORARIA* IN T_0 PLANTLETS

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Vitis vinifera, Vitreoscilla stercoraria, vh gene, photosynthetic activity, marker genes

The *vhb* gene of *Vitreoscilla stercoraria* expressing a haemoglobin with enhanced efficiencies as a response to oxygen stress has been transferred to microorganisms where has proved to achieve enhanced growth rate, increased synthesis of various compounds and improved efficiency of metabolic pathways. In transgenic *Populus* spp., plantlets expressing *vhb* did not reveal significant variations in developmental habits and biomass production (ZELASCO et al. 2006, Mol. Breed. 17, 201-216). Conversely, in tobacco, *Datura* and rice, *vhb* exogenous expression resulted in enhanced germination precocity, increased growth rate and improved synthesis of various compounds. Enhanced metabolic activity would be an appealing trait to be transferred, in particular in those plants species where increased cellular proliferation rate would be desirable. This is the case of grape, where gene transfer protocols are generally hindered by low morphogenesis. Besides, the *vhb* expression in grape plantlets would be an interesting tool for the selection of transgenics, if enhanced morphogenesis could be unambiguously related with outstanding traits.

Our study aims at assessing the applicability of *vhb* gene in grape, in the view of both improving morphogenesis and exploiting marker genes alternative to those conferring antibiotic resistance traits.

Gene transfer experiments were performed on embryogenic calli of *Vitis vinifera* 'Brachetto', using the pPLT7000 construct containing *vhb* and the *nptII* genes. After co-culture with *Agrobacterium*, putatively transgenic cultures were established using either supplemented or kanamycin-free media, in the view of comparing the phenotypes and the morphogenic potential of genetically modified plantlets with the wild-type control. In calli subcultured for 12 months, no differences in the proliferation rates have been noted between the cultures kept in the presence or in the absence of kanamycin. No plantlets were obtained from the putatively transgenic non-selection assay. Conversely, 20 potentially different lines of plantlets were recovered from the selection assay on kanamycin and from the control. Southern blot analysis resulted positive for exogene insertion. According to these results, the application of *vhb* as exogene able to combine useful traits with marker gene features seems to be unsuitable in 'Brachetto'.

In the view of assessing *vhb* effect, expressions of *vhb* and *nptII* genes were verified in 4 plants after amplification of the cDNA obtained from the total RNA and physiological analyses based on leaf oxygen emission, Fv/Fm ratio and electron transport rate were performed. A more efficient photosynthetic activity was measured in the transgenic plantlets, while chlorophyll content resulted similar to the one found in the wild-type controls. This finding might be related to an

increased biomass production trait. In the view of a more in depth analysis, a further investigation to be performed on *in vivo* plants after acclimation has been planned.

This research has been supported by the Autonomous Province of Trento (Project *EcoGenEtc.Com*). Authors wish to thank Ivana Gribaudo (IVV-CNR, Grugliasco Unit) for sharing with us the 'Brachetto' embryogenic calli, and Corrado Fogher (Plantechno, Piacenza) for providing us with the *A. tumefaciens* strain EHA 105 carrying the pPLT7000 vector.

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Poster Abstract – 7.70

GRAPE CLONAL CHARACTERIZATION TACKLED BY GENOME-WIDE ANALYSES

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Vitis vinifera, clonal variation, SNPlex™, Transposon Display, transposable elements

Grapevine (*Vitis vinifera* L.) is a long-living and woody perennial plant grown worldwide. Vegetative propagation over long periods favours the accumulation of mutations within individual genotypes, which exhibit altered phenotypes. Clonal selection as a procedure of crop improvement takes advantage of the identification of sports with agronomically and enologically important traits, which are vegetatively propagated resulting in new grape clones. Given the high economical value of clones, their identification is of great relevance and in addition it pertains issues of patenting and legal rights. While cultivar identification in grapevines is traditionally based on ampelographic descriptors and on microsatellite (SSR) profiles, clone discrimination is not possible with such tools. To allow true genetic identification of clones, molecular marker systems need to be developed ad hoc. Given the availability of the Pinot Noir (clone ENTAV 115) genome sequence, it is timely to use techniques exploiting the polymorphism information of unique coding and non-coding regions along with approaches based on specific genomic sequences of interest, such as DNA transposons and retrotransposons. Transposable elements (TEs), which possess the capability to change their genomic location, are potential source of mutations leading to clonal variation.

In this study we focus on the application of two genome sequence based approaches, SNPlex™ Genotyping System and Transposon Display, to tackle clonal characterization within six wine grape cultivars. We have analysed the state of 573 putative (electronic) SNPs, identified in coding and non-coding regions of the mentioned grape genome, in 141 genotypes. This sample set refers to 3 biological replicates (plants) of 47 clones (both registered and biotypes) belonging to the Pinot Noir, Pinot Gris, Pinot Blanc, Meunier, Teroldego and Gewürztraminer cultivars. The same set of clones was tested with 17 primers targeting specific regions (LTRs, LTR downstream or upstream, ORF) of TEs belonging to 6 families. Here we report preliminary results about the polymorphisms identified by different approaches enabling molecular characterization of clones within international and local grape varieties.

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Poster Abstract – 7.71

COMPARATIVE ANALYSIS OF 5S rDNA REPEATS IN WILD *VITIS* SPECIES AND GRAPEVINE

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grapevine, wild Vitis species, 5S rDNA

The genus *Vitis* consists of two subgenera, *Euvitis*, the most numerous which comprises species with 2n=38 and *Muscadinia* whose members have the somatic chromosome number of 40, with *V. rotundifolia* being the most known. This classification is based on a cytotaxonomic criterion which reflects a different evolutionary pathway within each group. The ancient basic chromosome numbers of the Vitaceae family are probably 5, 6 and 7. This suggests that *Vitis* species are ancient secondary polyploids, involving three basic sets in the combinations (6+7) + 6 = 19 and (6+7) +7 = 20 in. species with 2n=38 2n=40, respectively.

The aims of this study were to explore the genomic relationships between wild grapes and *V. vinifera* and achieve further evidence in favour of the polyploid origin of this genus. For this purpose the nucleotide composition of 5S rDNA sequences of *V. riparia*, *V. rupestris*, *V. labrusca* and V. *rotundifolia* were analyzed and compared with those previously isolated in grapevine cultivars. Sequences were isolated by PCR using two primers designed in such a way to isolate the complete NTS region and the adjacent transcribing regions.

The 5S rDNA of wild grapes showed a rather complex situation, similar to that described in *V. vinifera*. A long-repeat containing a NTS region of about 660 bp was found in all species. The spacers exhibited a remarkable variability in nucleotide composition both within and among species. On average, the identity within NTSs of wild species was comparable to that of each wild species with *V. vinifera*. A short-repeat with a spacer of nearly 332 bp, quite similar to that of grapevine cultivars was isolated in *V. rotundifolia*. The absence of PCR products in the remaining species indicated the need to verify the presence of this type of repeat by directly amplifying the short spacer with specific primers deduced from the short spacer sequences of *V. rotundifolia* and *V. vinifera*. The production of a fragment of nearly 250 bp, as expected from the position of the primers, demonstrated that the short repeat also occurs in *V. riparia*, *V. rupestris* and *V. labrusca*. A third type of repeat unit, exhibiting an NTS region of about 383 bp quite similar to a portion (330-660) of the long spacer, was only detected in *V. rotundifolia*.

The molecular organization of 5S rDNA found in wild *Vitis*, either with 2n=38 and 2n=40 chromosome number, appears rather similar to that previously described in grapevine. This reinforces the hypothesis that the species of the genus *Viti*s have common ancestors and a polyploid origin.

The presence of two distinct 5S rDNA repeats indicates that they evolved independently in different genomes. Instead, the difficulty of finding the short repeat in 2n=38 *Vitis* could be ascribed to possible differences in the organization of 5S rDNA tandem arrays between the two groups of

species. The 383 bp repeat found in *V. rotundifolia*, provides further evidence that specific 5S rDNA repeat variants may have originated following large deletions in the spacer region.

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Poster Abstract – 7.72

TRANSCRIPTOMIC ANALYSIS AND COMPUTATIONAL ANNOTATION OF GENES INVOLVED IN OLIVE FLOWER DEVELOPMENT

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Olea europaea, flower development, SSH, Gene Ontology

Olea europaea L. is a traditional tree crop of the Mediterranean basin. Despite the worldwide economical high impact factor, little is known on the physiological and molecular basis of ovule abortion and flower abscission, two processes that are thought to influence the plant fruit yield. Olive inflorescence, also referred as panicle, is composed by both perfect hermaphrodite and staminate flowers. Since fruit set is correlated to the proportion of perfect flowers within the inflorescence, the presence of staminate flowers is considered as an important yield-limiting factor in many crops. Furthermore, recent findings suggest that ovule abortion and the flower abscission might be under genetic control and influenced by both nutritional and environmental factors.

Two different approaches have been conducted to unravel the molecular network underlying the biological event.

The first deals with the identification of large sets of differentially expressed genes during the development of the flower and their computational annotation by means of different software.

Two forward and reverse subtractive hybridization libraries were constructed in order to isolate up- and down-regulated genes between two selected developmental stages in the cv Leccino. Our SSH approach originated a total of 1,127 clones which were analysed for the presence and amount of redundancy within and between libraries and computationally annotated. Library-specific cDNA repertories were analysed according to the three main vocabularies of gene ontology: cellular component, biological process and molecular function. BlastX analysis, GO terms mapping and annotation analysis were performed using the Blast2GO software, a research tool designed with the main purpose of enabling GO based data mining on sequence sets for which no GO annotation is yet available. Moreover, the olive flower-specific transcriptome dataset was used to query all known KEGG (Kyoto Encyclopedia of Genes and Genomes) metabolic pathways for characterizing and positioning of retrieved EST records within the flower development. On the whole, our approach led to the identification of sequences that proved to be significantly different in terms of abundance among the two developmental stages. Similarly, the integration of the olive sequence datasets within the MapMan platform for microarray analysis allowed the identification of specific biosynthetic pathways along with metabolic and regulatory maps useful for the definition of key functional categories in time course analyses for gene groups. Such analyses allowed the identification of sequences that were further investigated within cultivar characterized by a different fruit set behavior.

The second approach deals with expression studies of genes modulated during olive flowering. Two cultivars were compared, Dolce Agogia, which exhibits abundant flowering and

low fruit set, and Leccino, characterized by a stronger capacity of fruit set. The result obtained on *sus* expression were in agreement with the sucrose imported behaviour from the phloem in young fruits, confirming that carbohydrate supply is of fundamental importance for development and maturation of the floral organs and fruit setting. Gene expression studies on organelle's genes were also reported to better understanding their role in flower biological event.

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Poster Abstract – 7.73

SELF-INCOMPATIBILITY IN OLIVE (*OLEA EUROPAEA* L.)

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gametophytic self-incompatibility, SRNase, SLF, cyto-histology

Olive is among the oldest known cultivated trees in the world and it represents an important economic factor in the rural balance, particularly in Mediterranean basin, such as Italy. In spite of its cultural and economic importance few studies are carried out on reproductive barriers in this species. Particularly poor knowledge is available in literature about main problems of this species and its productivity. Although flowering is often abundant, poor fruit set, abortion of ovaries, self-sterility and self-incompatibility frequently occur. We focused our attention on the self-incompatibility, the less studied barrier, in order to shed light on the process as a whole. Comprehension of the genetic and molecular bases of this process should allow an increase of yield and a fundamental step forward the understanding of this very spread phenomenon in tree fruit plants. On the basis of the current knowledge, there are two big systems of self-incompatibility: sporophytic and gametophytic self-incompatibility (termed SSI and GSI, respectively). Within these groups, variants for incompatibility mechanisms are possible: one in SSI and two in GSI.

Despite the physiological importance and economical impact of this process in olive fruit yield, the definition of the compatibility behaviour is still not clear for many cultivars. From the literature, we hypothesize that olive is characterized by GSI, but no experimental data are now available and information is scanty. Through an accurate search of references, we decided to follow different experimental ways to investigate self-incompatibility in olive. We started our study by taking into account the GSI system by retrieving records in the NCBI databases belonging to species taxonomically related to olive (e.g. *Prunus* spp., *Petunia* spp., *Anthirrinum* spp., etc.) showing this trait. We designed degenerate primers on consensus sequences obtained by multiple alignments in order to isolate the candidate genes, that is the female and male genetic determinants, typical of this kind of incompatibility: the S-RNase protein, a ribonuclease, and the SLF (S-locus F-box containing) protein, involved in the SCF (Skp, Cullin, and F-box proteins) complex. As biological system, two putative self-compatible (Frantoio and Kalamata) and two self-incompatible (Leccino and Moraiolo) cultivars were analysed. Furthermore, the extensive sequencing of the transcriptome of the olive flower at different developmental stages is in progress by means of a 454 pyrosequencing approach. At the same time, cyto-histological analyses by means of stain-clearing and aniline blue staining of pistils are currently performed in self-compatible and self-incompatible cultivars to check the pattern of growth of pollen tubes through the stigma surface and eventually the transmitting tissue of the style until ovary. Overall results are presented and critically discussed.

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Poster Abstract – 7.74

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF NEW PUTATIVE OLIVE CULTIVAR OF MOLISE, ITALY

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molecular analysis, morphometric analysis, Nera di Colletorto, Noccioluta, statistical analysis

Olive cultivar classification is a primary concern for olive growers mainly because of the high number of synonyms and homonyms in existence, due to the similar morphology of the plants, which very often makes impossible to distinguish different genotypes. Two similar olive cultivar in Molise (commonly known as 'Oliva Nera di Colletorto' and 'Noccioluta') have been investigated, combining morphological and molecular data, to characterize them in two distinct cultivar. Fifty-seven trees were collected in the area of Molise (near Adriatic Sea): 23 and 34 individuals initially assigned by farmers respectively as 'Noccioluta' and 'Nera di Colletorto', were subjected to morphological and molecular analyses.

A comparative study of morphological traits in leaf, fruit and stone was based on the use of traditional linear measurement, a geometric-morphometric method (outlines), and volumetric data. Finally, mean weight and mean volume of fruits and stones were measured with a precision balance and a graduated cylinder. All recorded variables were subjected to statistical analyses: analysis of variance (ANOVA) was performed in order to individuate the morphological parameters with significant differences between means/groups; Principal Component Analysis (PCA) and cluster analysis (UPGMA) were performed using the selected variables from ANOVA, in order to evaluate the natural grouping without any prior hypothesis.

Eight nuclear microsatellite loci (SSR) were analyzed and allelic frequencies were processed to obtain genetic distance matrix among trees (Nei, 1972) by means of the GeneAlEx 6.0 program.

A hierarchical partition of genetic variation among and within groups was obtained by means of the analysis of molecular variance (AMOVA). Subsequently, we used PCA to order the location of the samples in relation to genetic distances, and cluster analysis (UPGMA) to produce a dendrogram. Statistical analysis individuated three significant differentiated groups and indicated high correlation between morphological and molecular data.

These results show clearly that molecular analysis supported the morphological results confirming microsatellites as a reliable tool very useful to integrate morphological investigation in phylogenetic studies. In fact, these three genetic groups found in this research were characterized by morphological differences in leaf, fruit, and stone.

These groups could be considered as three distinctive cultivar, but further investigations will be necessary for the quality and composition of the olive oil (chemical and organoleptic parameters). Finally, the improvement of olive oil production in this area of Molise could be encouraged by using this local cultivar, adapted and resistant to environmental conditions.

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Poster Abstract – 7.75

EXPLORING BIODIVERSITY OF SECULAR OLIVE TREES FROM APULIA REGION: A PATRIMONY TO EXPLOIT

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Secular Olive, AFLP, molecular characterization, genetic variability

Apulia region is, traditionally, the most important area for olive oil production in Italy. Nevertheless the wide olive germplasm collection there present is not completely identified and a number of valuable cultivars are not enrolled yet in the Register of olive cultivation. About 10% of the genetic patrimony is constituted, in particular, of the secular and monumental olive trees. A recently introduced regional law (4 june 2007 ns. 14) provides to exploit oil produced from secular olive trees through the definition of the special mention: "extravergin oil from the Apulia secular olive trees".

In this work, a first level investigation was carried out to study the genetic variability and the genetic relatedness inside a sample of secular trees and in comparison with known autochthonous cultivars. Leaf samples were collected from plants located in a large area between Bari and Brindisi provinces. Genomic DNA was extracted using a commercial kit (Sigma-Aldrich) and seven pair of AFLP primers were used to conduct selective amplifications. AFLP fragments were processed by 3130XL genetic analyzer (Applied Biosystems) and qualitative binary data processed using NT-SYSpc 2.2 software. A similarity matrix was obtained using Nei and Li coefficient and a dendrogram was constructed by means of the UPGMA algorithm. AFLP analysis of 21 accessions provided a total of 717 markers. An interesting cluster including all secular olive accessions emerged and a tight relationship was observed between secular olive accessions and Ogliarola Salentina and Cima di Mola cultivars. It is worthy of note that 'Ogliarola Salentina' is the most cultivated olive variety in Puglia and shows very similar morphological and agronomic traits to 'Cima di Mola' which led to considered this as a case of synonymy. The genetic relationships with secular olive germplasm seem to indicate a highly shared genepool, confirming their autochthonous origin. Genetic variability detected within secular olive germplasm by AFLP analysis indicates a good level of genetic variability such to justify a larger exploitation and biodiversity maintenance of Apulia secular germplasm. More information will be obtained with a more extensive sampling and molecular characterization of secular germplasm associated to qualitative oil analysis.

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Poster Abstract – 7.76

CHS INTRON 1 AS A MARKER OF VARIABILITY IN *OLEA EUROPAEA*

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Olea europaea, CHS, intron polimorphysms

Chalcone synthase (CHS) is the first enzyme of the flavonoid metabolic pathway. Comparison of *chs* genes sequences from different plant species reveals that the *chs* genes are structurally conserved and most of them contain one intron and two exons. CHS is encoded by a small multigene family in many plants. Until now, many *chs* genes have been cloned from monocot, dicot and some gymnosperm species; in *Olea europaea* two different genes have been recently identified.

In this work the intron 1 of *chs* genes was amplified in a selected number of Italian olive cultivars with different geographic origin and in the feral form *Olea europaea* var. *sylvestris.*

PCR amplifications carried out with the universal primers pair *chs*IntronFW/*chs*IntronREV (Strand et al., 1997 Mol Ecol., 6:113-118), produced a series of fragments that were cloned and sequenced. Sequence analysis showed different alleles of *chs A* gene; in particular we were able to found two different groups of alleles characterized by an high level of polymorphism between them.

Alleles of *Olea europaea* var. *sylvestris* belong to one of these groups that we named *Sylvestris like.* Sequence identity between the groups of alleles found in cultivated varieties and alleles found in the wild form was around 79%. It is worth noting the appearance in the *sylvestris-like* group alleles of a microsatellite poly-T with high degree of polymorphism.

The presence of the alleles belonging to *sylvestris-like* group in some cultivars suggested a possible hybridization between cultivated and wild forms.

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Poster Abstract – 7.77

SEARCHING FOR THE ORIGIN OF THE CULTIVATED APPLE

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SNP, domestication, phylogenesis, Malus

Researchers are divided about the wild ancestor(s) of the cultivated apple (*M. domestica*) and the time and geographic regions when and where the apple was domesticated. A first hypothesis assumes possibly multiple hybrization events between wild *Malus* species (*M. sieversii*, *M. sylvestris*, *M. orientalis, M. baccata* and others), which produced new apple types with large and sweet fruits, eventually brought to cultivation after the invention of the grafting technique. A second hypothesis, based on phytogeographic data and morphological affinities, recognized *M. sieversii* (a large-fruit wild apple species presenlty living in Kazakstan and west China) as the main, if not the only, ancestor of *M. domestica*. Recent molecular data comparing nuclear and chloroplast gene sequences did not provide a final answer. In this work we addressed the apple phylogenesis by assembling a panel of 74 accessions including the great most of the wild apple species and ten cultivars of *M. domestica*. For each accession, we sequenced 23 nuclear genes, evenly distributed on the genome. Based on the large dataset of about 700,000 bp, we computed multiple measures of the genetic similarity between accessions, at both the single nucleotide polymorphism and haplotype levels. We will report preliminary results about the phylogenetic relationships of *M. domestica* with its possible ancestors.

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Poster Abstract – 7.78

FINE-MAPPING OF THE *CO* LOCUS ON CHROMOSOME 10 CONTROLLING THE COLUMNAR HABIT OF APPLE (*MALUS X DOMESTICA* BORKH.*)*

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tree architecture, molecular markers, columnar, Malus domestica

The columnar tree habit in apple is caused by a dominant mutation originally identified in a spontaneous sport of McIntosh known as Wijcik. The columnar habit is characterized by short internodes, a thick stem and reduced plant height and branching. Most of the buds develop into short spurs, and long shoots are almost absent. As a consequence the tree will develop a natural narrow form that could be desirable in modern agriculture to assure a uniform light penetration, high density planting and a reduction of pruning interventions. The columnar habit was shown to be mainly determined by a single dominant gene (*Co*), mapping on the apple linkage group 10. To increase the *Co* genetic map resolution, we searched for SSR-type repetitive sequences linked to the *Co* locus, by screening the Golden Delicious genome sequence made available by the sequencing project in progress at FEM-IASMA. Based on such analysis, SSR markers were designed and tested onto three different populations of 170, 130 and 70 individuals respectively derived from the crosses “Golden x Wijcik”, “Goldrush x Wijcik” and “Galaxy x Wijcik”. Ten new SSR markers closely linked to the *Co* were eventually mapped, enabling to narrow down its physical position within a 800-kb region. Among the genes annotated in this region, we highlighted seven putative candidate genes possibly involved in the control of tree branching.

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Poster Abstract – 7.79

DEVELOPMENT AND CHARACTERIZATION OF MICROSATELLITES MARKERS FROM *JUGLANS REGIA* L.

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microsatellites, Persian walnut, isolation, characterization

Simple Sequence Reapeats (SSRs) were isolated from two microsatellites GA/GT enriched libraries from Persian walnut (*Juglans regia* L.). After screening, 10 selected microsatellites loci were characterized and evaluated on 36 accessions from National and International germplasm. All primers pairs produced an amplification product of the expected size and detected high polimorphism among the analysed samples. These SSR markers are expected to be an effective tool for performing MAS (Marker Assisted Selection) and saturating genetic maps.

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Poster Abstract – 7.80

MULTIPLE ORIGIN OF CULTIVATED EUROPEAN HAZELNUT (*CORYLUS AVELLANA* L.) ASSESSED BY CHLOROPLAST MICROSATELLITE ANALYSIS

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chlorotype, cpSSR, cultivar diffusion, domestication centres, genetic diversity

The present-day distribution of European hazel (*Corylus avellana* L.) was established about 7,000 years B.P. as result of a postglacial recolonization process. Nut dispersal was mainly by animals and human migration. Mesolithic tribes could have aided, intentionally or more likely accidentally, the spread of hazel. The place and time of hazel domestication is not clear, although it was already cultivated by the Romans. The common opinion is based on a statement of Pliny the Elder (23–79 A.D.) in the work *Naturalis Historia* that the hazelnut came from Asia Minor and Pontus (north coast of Turkey). On the basis of this assertion, the accepted general idea is that hazelnut cultivation was brought to Italy by the Greeks. However, the existence of morphological differentiation between cultivars from eastern and western ends of the Mediterranean basin suggests the existence of different genetic contribution from local populations or multilocal selection and domestication of wild genotypes. In order to tackle this issue, the genetic relationships between chlorotype variation and distribution were analysed in 525 samples of cultivated (185) and wild (340) genotypes collected in most of the areas where the specie is growing (o mettere il numero di località). The results suggest the existence of at least two important origins for the cultivated germplasm: one in Europe and another in Turkey-Transcaucasus-Iran area. Minor centres of domestication were also the Balkans and Russia.

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Poster Abstract – 7.81

DIFFERENTIAL GENE EXPRESSION ANALYSIS TO INVESTIGATE SPOROPHYTIC SELF-INCOMPATIBILITY IN *CORYLUS AVELLANA* L.

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hazelnut, S locus, female determinant, differential display, peroxidase

The European hazelnut (*Corylus avellana* L.) is a monoeciuos tree and exhibits sporophytic self-incompatibility (SSI). Self-incompatibility (SI) is a genetic system that prevents the self-fertilization, allowing the pistil to reject the pollen of genetically close individuals and limiting therefore the possibility of crossing between individuals. Therefore the choice of pollinizers is critical for assuring good and constant yield; SII in this species is therefore an important aspect to be considered in breeding programs for orchard planting.

Sporophytic self-incompatibility is controlled by a single multi-allelic locus, the S locus. The involved molecular mechanisms are well known only in *Brassicaceae*, although SSI is present also in *Asteraceae*, *Betulaceae*, *Caryophillaceae*, *Convolvulaceae* and *Sterculiaceae.*

Studies on gene regulation of fertility, pollination and fertilization in hazelnut are very few; therefore with this research we propose to contribute to the knowledge about the genetic bases of flower biology of hazelnut.

At a first stage, homologies between the S locus of *Brassica* and *Corylus* were sought using degenerated primers, but without success.

Therefore, the Differential Display technique was applied for the study of the female determinant of self-incompatibility. Two developmental stages of female flower buds were compared: before styles emergence and at full bloom. Partial sequences of genes that may be involved in the mechanisms of pollen recognition, signal transduction and flower development were isolated and identified after blasting in TIGR and NCBI databases. Among the isolated sequences, one was identified so far as being from a putative peroxidase gene that was eventually fully characterized and is described as a class III peroxidase.

Research carried out within the project "Proteomic and gene expression differential analysis for studying sporophytic self-incompatibility in hazelnut" PRIN- MIUR 2007

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Poster Abstract – 7.82

GENETIC VARIABILITY AND DIVERGENCE AMONG ITALIAN POPULATIONS OF SILVER FIR

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Abies alba, genetic differentiation, genetic variability, microsatellites, Regions of Provenance

Forest trees are exposed to many stress factors, most of which are due to human activities: pollution, climate change, habitat fragmentation. In order to survive these threats, and to persist over time, a high adaptive potential is needed: this is mainly determined by the within-species genetic diversity. Programmes aimed at the conservation of forest genetic resources should address the issue of maintenance of this diversity.

To this end, knowledge of genetic variation are of the utmost importance. The analysis of morphological and phenological variation, i. e. adaptative traits, requires long-lasting and multi-site trials, very difficult to establish. Molecular markers are now available and can provide us with the relevant means to acquire information on the genetic structure of populations and to study the pattern of distribution of within-species variability. In particular, simple sequence repeats (also known as microsatellites) are commonly used in genetic studies of plant populations.

The purpose of this study was the evaluation of neutral DNA markers (microsatellites) as a tool to study genetic variability distribution of silver fir (*Abies alba* Miller) in Italy, and to group populations according to their genetic similarity. 42 natural silver fir populations, representing the locations where the species grows in Italy, were sampled and DNA was extracted from young leaves. Nine microsatellite primer pairs were used to detect genetic variability. They were highly polymorphic, displaying a high number of alleles and a wide size range of PCR products. Levels of within and among populations variability were estimated and genetic differentiation was calculated. High polymorphism was found within populations, as on average more than 10 alleles were observed per locus and the probability that two randomly sampled alleles in a given population were different was almost 70%. Observed heterozygosity was lower than expected one, causing a significant positive mean inbreeding coefficient. This can be due to the presence of null alleles, so that some heterozygotes are mistaken for homozygotes. However, it is not possible to exclude the occurrence of inbreeding, although the mating system of the specie should account for crossing. Additionally, the ecological features of the collection sites were analysed (mainly concerning climatic conditions and soil characteristics) and homogeneous regions were defined. Lastly, patterns of genetic and ecological variations were compared, allowing us to identify areas that are both ecologically and genetically homogeneous.

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Poster Abstract – 7.83

EFFECTS OF GENETIC TRANSFORMATION ON *cry* TRANSGENIC LINES OF POPLAR

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cry *gene, poplar transgenic lines, Agrobacterium tumefaciens*

Problems, such as the impact on biodiversity and the potential escapes of the trans-genes into wild populations can be amplified in the case of long-lived forest species. Therefore, studies of possible environmental impacts of transgenic forest plants (poplar) in the forest environment are important. Few studies have been published considering the alteration of the transgenic host plant genome for the possibility that transgenes can also cause pleiotropic, often undesirable alterations in plant metabolism and physiology. Other studies carried out so far, concerned the use of transgenesis to obtain poplar mutants for genomic studies or poplar breeding just for an economic purpose.

The screening of the transgenic plants carrying the *cry* gene is in course, and the evaluation of the copy number as well as expression of the inserted gene is under evaluation.

The final aim is to unravel possible pleiotropic metabolic effects in the transgenic trees following *cry* gene expression in *P. alba* and *P. tremula* x *P. tremuloides* transgenic lines.

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Poster Abstract – 7.84

GENETIC VARIABILITY OF *QUERCUS PUBESCENS* POPULATIONS FROM NORTH-WESTERN ITALY AND DIFFERENTIATION FROM OTHER OAK SPECIES BELONGING TO *ROBUR* SECTOR

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Pubescent oak, genetic differentiation, genetic variability, microsatellites, gene introgression

Pubescent oak (*Quercus pubescens* Willd.) is relatively common in north-western Italy, where it grows mainly in hilly areas and in the basal sector of alpine valleys. The species is phylogenetically related to sessile oak and English oak and gene flow between taxa is possible.

In this work we have analysed morphological and molecular variability to describe the genetic variation and differentiation within and among several natural populations of pubescent oak.

About twenty morphological traits of the leaves and eight microsatellite markers were used to detect genetic variability. The latter were highly polymorphic, displaying a high number of alleles and a wide size range of PCR products. Levels of within and among populations variability were estimated and genetic differentiation was calculated.

Since a mixed stand, where all three species are present, were included in the study, the effect of intraspecific gene flow on the genetic structure of *Q. pubescens*, *Q. petraea* and *Q. robur* has also been evaluated, and general *versus* local differentiation has been quantified.

The data obtained are of basic importance for the characterisation of genetic resources and for the adoption of practical and effective measures for *in situ* preservation of biodiversity. Genetic variability is in fact considered the most important factor responsible for adaptive capacity, and therefore for survival under spatial and temporal variation of the environment. We suggest using the results of this work also for the identification of the most valuable stands for production of high quality seeds.

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Poster Abstract – 7.85

GENETIC EFFECTS OF CHRONIC HABITAT FRAGMENTATION REVISITED: STRONG GENETIC STRUCTURE IN A TEMPERATE TREE, *TAXUS BACCATA* L. (TAXACEAE), WITH GREAT DISPERSAL CAPABILITY

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forest fragmentation, gene flow, microsatellites, spatial genetic structure, Taxus baccata

Tree species are thought to be relatively resistant to habitat fragmentation due to their longevity and their aptitude for extensive gene flow, though recent empirical studies have reported negative genetic consequences, in particular after long-term habitat fragmentation in European temperate regions. Yet the response of each species to habitat loss may differ greatly depending on their biological attributes, in particular seed dispersal ability. In this study we used demographic and molecular data to investigate the genetic consequences of chronic habitat fragmentation in remnant populations of *Taxus baccata* in the Montseny Mountains, northeast Spain. The age structure of populations revealed demographic bottlenecks and recruitment events associated with exploitation and management practices. We found a strong genetic structure, both at the landscape and within population levels. We also detected high levels of inbreeding for a strictly outcrossing species. Chronic forest fragmentation resulting from long-term exploitation in the Montseny Mountains seems the most plausible explanation for the strong genetic structure observed. Our results support the view that, contrary to some predictions, tree species are not buffered from the adverse effects of habitat fragmentation, even in the case of species with a high dispersal potential.

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Poster Abstract – 7.86

DEMOGRAPHIC AND ADAPTIVE CONSEQUENCES OF LONG-RANGE COLONIZATION IN ALEPPO PINE

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demography, selection, genetic drift, colonization, local adaptation

The evolutionary outcomes of range expansion/contraction depend on the biological system considered and on the interactions among the evolutionary forces in place. In this study, we examine the consequences of long-range colonization on the demography and local adaptation of a widespread Mediterranean conifer (*Pinus halepensis* Mill.). To that aim, we used cpSSRs and coalescence modelling of nuclear genes to infer the demographic history of natural populations covering the species range. Ten drought-response candidate genes were then examined for their patterns of polymorphism and tested for selection considering plausible demographic scenarios. Our results revealed a marked loss of genetic diversity from the refugial core region, located in Greece, to its western range, as well as molecular signatures of intense and recent bottlenecks. Moreover, we found an excess of derived polymorphisms in several genes sampled in the recently colonized range but not in the refugial area, a potential result of the action of natural selection during long-range colonization. Wide-range expansions/contractions of forest trees are accompanied by strong selective pressures, resulting in distinct evolutionary units, a knowledge that is of crucial importance for the conservation and management of forests in the face of the current process of climate change.

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Poster Abstract – 7.87

DEVELOPMENT OF A NOVEL SET OF EST-SSR MARKERS IN THE GENUS *TAMARIX* AND THEIR APPLICATION IN A POPULATION FROM SARDINIA

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Tamarix, EST-SSR, Genetic variability, Abiotic stress

Global warming is expected to result in a general decrease of precipitations and an overall increase of extreme weather conditions, with a consequent decline of Mediterranean forests. *Tamarix* plants are resistant to abiotic stresses, as they thrive in zones where drought, soil salinity, and high temperature are regular phenomena. The natural survival capacity and genetic diversity of *Tamarix* plants can be harnessed to sequester CO_2 from atmosphere, by increasing their plantation in presently unutilized areas. Italian coastal zones could represent a source *Tamarix* germplasm adapted to stressing habitats but there is not any information about the genetic structure of the Italian germplasm. Moreover, the taxonomy of the genus *Tamarix* is one of the most troublesome among the angiosperms. In fact, the species cannot be distinguished at vegetative state and some morphological traits used in species identification could vary from season to season on the same individual. A good knowledge of the natural genetic resources and variability of *Tamarix* is the basis for the selection of tolerant genotypes for the recovery of degraded areas. Molecular markers are fundamental tools both for studying plant biodiversity and for breeding programmes. Unfortunately few molecular markers have been developed for the genus *Tamarix*.

The aim of this work is the development of an innovative methodology for genetic characterization of the genus *Tamarix* with special regards to the Italian species. Our approach is based on the publicly available sequences emerging from large-scale EST sequencing projects, which offer a potential source for a quick and cheap development of new markers, named EST-SSR.

A mixed population of *T. gallica* and *T. africana* around Baratz Lake (Sardinia) was chosen for our study. The site is characterized by the presence of salty water, and high variability in water availability; so, plants are subjected to alternate periods of drought and soil salinization, and flooding.

EST sequences derived from four *Tamarix* species were acquired separately, and assembled to eliminate redundancy. These non-redundant sets were screened for the presence of microsatellites and primers flanking the SSR region were designed. A set of eight polymorphic *loci* were selected and analysed on 31 plants, which were sampled around Baratz Lake without any regard for species identity. DNA was extracted using a modified CTAB method and PCR reaction was conducted with fluorescent labeled primers following M13-tailed technique. The EST-SSR markers showed cross amplification in the two species present in the site of study, moreover, intraspecific and interspecific polymorphism was observed. The new set of polymorphic EST-SSR can be used as additional tool for taxonomy comparing our unknown samples to other vouchered *Tamarix* accessions.

Our results provide a first contribution to the genetic diversity of Italian species. Moreover, the amount and distribution of genetic variability of *Tamarix* plants from lake Baratz will clarify the ecological role of *T. africana* and *T. gallica* in a site characterized by hard environmental conditions, and offer information about genetic basis of abiotic stress tolerance in *Tamarix*.

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Poster Abstract – 7.88

SOLVING THE SEX DILEMMA IN TRUFFLES BY A GENOME-BASED APPROACH

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Tuber melanosporum, mating type, sexual reproduction, SSR (simple sequence repeat)

Tuber melanosporum and *T.magnatum* are ectomycorrhizal ascomycetes producing edible ascocarps, known as black and white truffles, respectively. These species are highly praised worldwide for their intense bouquet, and their market is steadily expanding. Yet, most of the ecological and biological aspects that govern truffle life cycle remain still largely unknown. In particular, gaining direct evidence on the mating strategies that control the production of their prestigious ascocarps is hampered by the impossibility to mate these fungi under controlled conditions. Thus, truffle sex is a long lasting dilemma for mycologists.

Caryological and molecular data have conveyed the idea that truffles strictly self and have a prevalent diploid/dikaryotic phase in their life cycle (1,2). In departure from this view, we have recently produced SSR-based evidence for the prevalence of the haploid phase in the truffle life cycle and the occurrence of outcrossing in both *Tuber melanosporum* and *T.magnatum*. (3-5). Despite this new finding, however, the hypothesis of selfing in *Tuber* spp. is far from being settled. Indeed, the possibility that truffles are homothallic and facultative outcrossing species, switching from one reproductive tactic (selfing) to the other (outcrossing) according to external stimuli is still plausible (6).

The sequencing of *T. melanosporum* genome (*Tuber* Genome Consortium: http://mycor.nancy.inra.fr) is in progress (7, 8). Within the frame of this bilateral French-Italian genome project we have studied the structure and organization of mating (MAT) genes in this species.

Here we show that *T. melanosporum* is an heterotallic fungus: the structure and organization of *T. melanosporum* MAT genes highly resemble those of other heterothallic ascomycetes with two MAT idiomorphs harbored by different mycelial strains.

This finding is practically very relevant in that it paves the way to a profound re-evaluation of *T.melanosporum* cultivation and conservation strategies. Not only the sequencing of *T.melanosporum* genome has allowed us to resolve the dilemma concerning the sexuality in this species but also it provides mycologists with genetic tools to successfully tackle this critical issue in other *Tuber* spp.

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Poster Abstract – 7.89

CHARACTERIZATION OF YEAST-LIKE STRAINS ISOLATED FROM DETERIORATED AREAS OF TWO MARBLE STATUES

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yeasts, microcolonial fungi, AFLP

One of the most common cause of blackening on artistic marbles and limestones is their colonization by the so-called dematiaceous fungi, a group of darkly pigmented fungi including black yeasts, meristematic and microcolonial fungi. These microorganisms share common growth and morphological features, even if they can be phylogenetically distant. In many cases they form cauliflower-like microcolonies on and in rocks incrusted with melanins deposited in their cell wall, giving them a dark, blackish brown appearance. Some authors consider them the most harmful microorganisms for stones. The cause of damage seems due to a physical attack with cracks and fissures formation rather than to chemical dissolution of minerals.

Here we report a blackening biodeterioration case of two very valuable marble statues outdoor exposed in Piazza della Signoria, Firenze (Italy): the "Ratto delle Sabine" realized in 1583 by Giambologna and the "Copia del David" realized in 1910 by Luigi Arighetti. Both statues showed dark-grey spots widespread in some areas of their surface. From the superficial marble particulate sampled in different times from the deteriorated areas, three black fungal strains were isolated called M4 (from the "Ratto delle Sabine"), D1 and D3 (from the "Copia del David"). Their growth and morphological characteristics were very similar; microscopical analysis showed in each case a yeast-like morphology and colony morphology resembled that of microcolonies observed by ESEM in the sampled marble. Moreover, two pink-red yeasts called RS (from the "Ratto delle Sabine") and D (from the "Copia del David") grew from the marble particulate as colonies strictly close to those of black fungi. All the isolated strains were characterized by molecular methods based on small subunit and internal transcribed spacer regions of rDNA. To better investigate the phylogenetic relationship among black as well as red yeasts, they were also characterized by AFLP analysis.

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Poster Abstract – 7.90

CYTOGENETIC INVESTIGATIONS IN CATTLE EXPOSED TO DIOXINS AND FISH-MAPPING OF *ARNT*, *CYP1B1* AND *CYP1A2* IN BOVIDS

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cattle, dioxin, SCE, chromosome fragility, FISH

Dioxins is a large family of chlorinate compounds, between polychlorodibenzodioxins (PCDDs) and related substances including polychlorodibenzofurans (PCDF) and dioxin-like polychlorobiphenyls (DL-PCBs) with high toxicity. These chemicals can enter the food chain as the result of a number of industrial processes, waste incineration or illegal waste burning. Cytogenetic tests could be useful to reveal the presence of such mutagens in the food chain by simply monitoring food producing species. In previous studies we studied sheep exposed to dioxins and found a pronounced chromosome fragility in exposed herds, compared to unexposed ones (control) by using both SCE and chromosome abnormality (AC) test. In this study we applied both AC (gap, chromosome and chromatid breaks, fragments, aneuploidy) and SCE-test in cattle reared in dioxin-contaminated areas of Piedmont region. Peripheral blood cultures were performed in samples from three groups (16 animals per group) of dairy cows, of which two (hybrids between Piedmont-Valdostana breeds) showing average milk values of dioxins over (18.56 and 8.56 pg/g of fat as WHO-TEQ) than those permitted (6.0 pg/g of dioxins+furans+PCBs as WHO-TEQ) and the results were compared with samples from dairy cows (Valdostana breed) showing lower levels of dioxins (1.75 pg/g of fat as WHO-TEQ) than those permitted used as control. A significant ($P<0.01$) higher chromosome fragility was found in exposed cattle (both two groups) compared to that of control. In addition, we have comparatively FISH-mapped three loci (*ARNT, CYP1B1 and CYP1A2)*involved in the dioxin metabolism in cattle, river buffalo and sheep chromosomes by using simultaneous visualization of RBPI-banding (*R-bands by using late incorporation of BrdU and Propidium Iiodide staining*) and FITC-signals and found that these loci were conserved in homologous chromosomes and chromosome bands of the three bovid species.

The research was supported by National Research Council of Rome, Italy, DG.RSTL.083.001 and DIOSSOPOLI project, Piedmont Region, Agricultural Direction, Turin

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Poster Abstract – 7.91

CHIANINA AND MARCHIGIANA BREEDS: EFFECT OF SOME SNPs IN CANDIDATE GENES FOR MEAT PRODUCTION

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SNPs, beef production, Chianina, Marchigiana

At present, Breeders Associations are asking for some new selection strategies mainly because current methods require long time to achieve a good accuracy; therefore marker assisted selection (MAS) can be considered as a new efficient tool.

A preliminary bibliographic survey allowed us to identify 26 SNPs putatively correlated with beef cattle growth and meat production traits.

DNA samples from 249 Marchigiana and 365 Chianina sires, tested in performance test, were sent to KBioscience to be genotyped. Only 15 SNPs belonging to 11 genes showed to be polymorphic in both the breeds. For some of them an association with production traits was also observed.

SNP6 (GDF8) showed, both in Chianina and Marchigiana, an heavier weight at birth, an higher muscolarity score and a bigger dimension genetic index (100±10) associated with the genotype AA (45.43 kg – 424.21 – 105.66) vs AT (43.65 kg – 400.84 – 103.91) and TT (44.64 kg – 403.94 – 104.87).

Similar results were observed in Marchigiana for genotype GG vs AG and GG in SNP9 (GHRL) and in Chianina for genotype TT vs CT and CC in SNP16 (IGF2).

These results allow us to suppose some effects of the investigated SNPs on beef traits both in Chianina and Marchigiana breeds; therefore if these results will be confirmed on a larger dataset, these SNPs could be suitable to select the calves to be tested in the performance station.

The research was supported by SELMOL project (MiPAAF).

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Poster Abstract – 7.92

IN SILICO/WET APPROACH TO IDENTIFY REFERENCE GENES FOR EXPRESSION ANALYSIS IN **WATER BUFFALO**

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gene expression analysis, reference genes, normalization, RTqPCR, Bubalus bubalis

In gene expression analysis, a key step to obtain informative data from RT qPCR assay is the normalization, that is usually achieved by means to correct the abundance of the gene of interest against that of an endogenous reference gene transcript. However, the finding of such reference genes, ideally expressed in a stable way in multiple times and in different experimental conditions, is a non-trivial problem. In particular, for gene expression studies in riverine buffalo, no reference gene has been proposed until now. In our work, a publicy available *Bos taurus* ESTs database has been screened to identify candidate housekeeping genes. In order to evaluate the potential of such candidates for their use as normalizers in buffalo gene expression analysis, a lab bench based test has been done, in which the expression stability of these genes has been evaluated on a panel of buffalo tissues and organs. A comparison between non-normalised gene expression data and the data after normalisation with the proposed reference genes and a "common" housekeeping gene like beta-actin has been done.

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