Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.01

ASSESSMENT OF GENETIC DIVERSITY OF EUROPEAN EMMER WHEAT POPULATIONS BY EST-SSR, ISSR AND SSR MOLECULAR MARKERS

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Triticum dicoccum, EST-SSR, ISSR, SSR, variability

Emmer wheat has been rediscovered in the last decades due to its characteristics. Moreover, its wild behaviour made its cultivation in the marginal lands interesting from the economical point of view. A total of 38 emmer wheat (*Triticum dicoccum*) accessions, collected around Europe from: Germany (15 accessions), Austria, Italy (8 accessions), Spain (3 accessions), Slovakia (2 accessions), and Israel, were evaluated utilizing both agro-morphological characteristics and molecular markers.

The agronomic traits evaluated were: vernalisation response, winter hardiness, date of heading and flowering, lodging, plant height at harvest, and resistances against powdery mildew (*Erysiphe graminis*), leaf rust (*Puccinia recondita*), and yellow rust (*Puccinia striiformis*). Quality control was also performed measuring the protein content, gluten quality and quantity, and a baking test was performed.

The molecular evaluation was carried out utilizing 12 microsatellite (EST-SSR and SSR) and 6 ISSR primers for a total of 107 loci analysed. Some of the EST-SSR markers have homology to genes of known function such as: ABA induced protein, PM-19, alcohol dehydrogenase, ADP/ATP translocator gene, and CERI/like 3' gene.

Mean 1000 kernel weight ranges from 31.6 to 39.0 g for winter emmer accessions and from 22.9 to 42.6 for spring emmer accessions. Almost all spring emmer accessions showed resistance to powdery mildew. The protein content of both winter and spring emmer was significantly effected by environment and genotype ($P<0.0001$). Measurements of wet gluten content in emmer accessions revealed high values, ranging from 37.0 to 56.6 %.

The molecular markers revealed a great Nei genetic distance between the analysed accessions. The expected heterozygosity and the variance between accessions were high, indicating an equal distribution of the alleles and the presence of great differences in the analysed material. The molecular analysis showed different pictures if in the analysis were considered only the microsatellite or both kind of markers. The microsatellite were able to discriminate between country of origin, even if some miss-classification was present. On the other hand, no consistent clusters were obtained considering winter versus spring accessions.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
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ISBN **88-900622-6-6**

Poster Abstract - C.02

WILD EMMER AND SPELT EUROPEAN WHEAT: AGRONOMICAL AND GRAIN QUALITY TRAITS EVALUATION

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Triticum dicoccum, Triticum spelta, grain yield, quality

Organic agriculture and health food products have been gaining increasing popularity that has led to a renewed interest in hulled wheat species such as emmer (*Triticum turgidum* spp. *dicoccum*) and spelt (*Triticum aestivum* spp. *spelta*). The description of agronomically important and useful characteristics is an important prerequisite for effective and efficient use of germplasm collections in breeding programs. In spite of the importance of the Italian landraces only limited agromorphological characterization data are available. The objective of this study was to estimate agronomical and grain quality characteristics of some Italian emmerand European spelt wheat. A total of 30 wild emmer and spelta accessions consisting of 20 emmer populations, advanced breeding lines and variety originated from Italy and 10 spelt wheat genotypes originated and still cultivated in Central and North Europe were examined under low input conditions. The study was conducted for three successive years (2001/2002, 2002/2003, 2003/2004) at one location of Southern Italy (Foggia). The crop cycle was from November to end June. In both seasons, each genotypes was planted in three replicates in a randomized complete block design. The entries were characterized for qualitative [grain protein content as N * 5.7 (%), gluten index and yellow index colour measured by CIE colour values (L*a*b*)] and quantitative traits [grain yield (t ha^{-1}), thousand grain weight (g), test weight (kg hl^{-1})]. The results showed a large genetic variability for many agronomically important traits and, even thought the genetic materials showed inferior bread- and pasta-making performance, several genotypes exhibited some potential to perform good pasta and bread thanks to processing technologies (allowing the use of flour and semolina for making biscuits and pasta) and marketing strategies (emphasizing its low input cultivating practices).

Acknowledgement
This work was supported by MiPAF special grant “Risorse genetiche di organismi utili per il miglioramento di specie di interesse agrario e per un’agricoltura sostenibile”.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.03

ON-FARM CONSERVATION AND ENHANCEMENT OF LOCAL DURUM WHEAT GENETIC RESOURCES IN ETHIOPIA

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durum wheat, farmer selection, landraces, re-introduction

In Ethiopia, tetraploid wheat (*Triticum turgidum* L.), mainly represented by durum wheat (*Triticum turgidum* var. *durum)* has been under cultivation since ancient time. It is traditionally grown by smallholder farmers on heavy black clay soil of the highlands between 1,800 and 2,800 m a.s.l.

Using locally available durum wheat resources and their own knowledge and traditional practices, farmers have been developing a broad range of genetically diverse durum wheat landraces fitting the highly varied existing micro-environments for soil, water, temperature, altitude and slope characteristics. Multiple goals were motivating farmers' communities in their selection activities, such as maintenance of stable production systems of low-input agriculture in marginal environments, maximisation of output under adverse farming conditions, and meeting of the dynamically evolving market demands.

Despite the great advantages of using local adapted germplasm, durum wheat landraces diversity is presently subject to serious threat of genetic erosion and irreversible losses due to its replacement with new, exogenous, high-yielding genetically uniform cultivars of bread wheat. Moreover, no effective results have been achieved by the National Durum Wheat Improvement Programme which has mainly relied on the delivery of modern, high yielding durum wheat varieties selected from advanced breeding materials provided by international research programmes. As a result, the diffusion in the farmer community of these newly proposed materials has been limited, due to the unavailability of seeds, to the high cost of the required external inputs, and especially to the limited adaptability and stability of the new varieties to the adverse and varied farming conditions when compared to the locally adapted farmer varieties.

Together with the genetic material loss, there has been the dispersion of associated farmer's knowledge on management practices and traditional uses of the products, which lead to limiting farmers' capability to face adverse climatic conditions, as well as new challenges posed by quick evolving societies and markets. This is particularly true in marginal production areas where small-scale farmers and communities could be further more exposed to food insecurity and poverty.

In this contest, the Istituto Agronomico per l'Oltremare, technical and scientific body of the Italian Ministry of Foreign Affairs, is supporting the local NGO Ethio-Organic Seed Action (EOSA) in its programme of re-introduction and enhancement of local durum wheat farmer varieties in Ethiopia.

The aim of the programme is to restore a community-based management and improvement of durum wheat landraces, according to traditional and new market requirements, i.e. the growing national pasta making industry. A renewed partnership of farmers' communities with the agricultural research centres is

promoted, toward ensuring mutual understanding and strict cooperation for production increase and environmental conservation.

The poster describes the different phases implemented by the programme starting from the re-introduction of durum wheat accessions stored in the national genebank to the selection and multiplication in farmers' fields. Preliminary results on field performance and quality assessment of 10 promising improved landraces. are presented. Moreover, the genetic characterisation of durum wheat landraces by electrophoresis of gliadin is presented and implications discussed.

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ISBN **88-900622-6-6**

Poster Abstract - C.04

USING A DURUM WHEAT GERMPLASM COLLECTION SUITABLE FOR GENE DISCOVERY VIA ASSOCIATION MAPPING: CHROMOSOME REGIONS FOR RESISTANCE TO SOIL-BORNE CEREAL MOSAIC VIRUS

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durum wheat (Triticum durum Desf.), linkage disequilibrium (LD), association mapping, QTLs, soil-borne cereal mosaic virus (SBCMV)

A collection of 134 durum wheat accessions chosen to sample the genetic variation present in the major cultivated gene pools has been assembled for genetic association study and allele mining purposes. The collection has been characterized molecularly with SSR markers to evaluate the pattern of long-range linkage disequilibrium (LD) and the presence of population structure (Maccaferri *et al.*, 2005). The phenotypic variation present in the collection is also being studied: various morphological descriptors and agronomically valuable traits have been recorded in comparative field trials carried out during the last three growing seasons. Up to now the collection have been characterized with 100 SSR markers, chosen on the basis of their map position and molecular information content.

A subset of 113 accessions were evaluated in two consecutive seasons (2003 and 2004) for resistance/tolerance to soil-borne cereal mosaic virus (SBCMV), a disease that affects bread and durum wheat in several regions of the world and especially in the north and centre of wheat growing areas in Italy. SSR data were subjected to the marker/phenotype association analysis after accounting for the population structure. A number of chromosome regions (e.g. on chromosome arms 4AL, 4BS, 5BL) showed a significant association with symptom severity and/or virus concentration in both years. These chromosome regions represent good candidates for harbouring valuable resistance genes/QTLs in durum wheat and are now being subjected to a more refined genetic analysis to validate their genetic effects on SBCMV. The results herein presented indicate the feasibility of utilizing association mapping for identifying chromosome regions that influence agronomic traits in durum wheat.

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ISBN **88-900622-6-6**

Poster Abstract - C.05

IDUWUE: A PROJECT FOR THE IMPROVEMENT OF WATER-USE EFFICIENCY AND DROUGHT TOLERANCE OF DURUM WHEAT

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durum wheat, Triticum durum, drought tolerance, water-use efficiency

In the Mediterranean basin, durum wheat is mainly grown in drought-prone areas. Therefore, improving water-use efficiency and tolerance to drought represent major breeding goals. IDuWUE (Improving Durum wheat for Water Use Efficiency and yield stability through physiological and molecular approaches) is a collaborative project among Research Centres in Italy, Spain, Morocco, Tunisia, Syria and Lebanon funded by the European Union aimed at investigating the genetic variability for water-use efficiency (WUE) and yield stability in durum wheat genotypes grown in the Mediterranean drought-prone areas. A number of morpho-physiological traits (e.g. early vigor, flowering time, leaf rolling, number of fertile tillers, etc.), WUE, WUE-related traits (e.g. carbon isotope discrimination, canopy temperature, chlorophyll fluorescence, etc.), yield and its components are being investigated on a RIL population (249 lines) and a collection of ca. 190 durum wheat accessions during the first year of the project in field trials carried out under irrigated and rainfed conditions. The results of the QTL analysis carried out on the mapping population will be integrated with an LD association study performed on the collection of accessions. In this respect, the population structure has been preliminarily estimated with AFLPs and will be further investigated with SSRs. Recent work has indicated the presence of a high level of LD in durum wheat (Maccaferri et al., 2005, Molecular Breeding, 15:271-289). The molecular and phenotypic results so far obtained on the collection of accessions will be presented and discussed.

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ISBN **88-900622-6-6**

Poster Abstract - C.06

FINGERPRINTING WHEAT VARIETIES FOR BREEDING PURPOSES

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Triticum durum, Triticum aestivum, molecular markers, pure line identification, linkage disequilibrium

Modern wheat varieties are thought to display quite low levels of gene pool variation because of the high selective pressure applied in breeding programs. The genetic diversity of durum wheat and bread wheat elite germplasm has been traditionally estimated on the basis of morphological and quantitative traits, disease resistances, gliadin proteins and only recently by molecular markers. In the last decade, RFLP and PCR-derived markers have been extensively applied in wheat genetics, not only for the construction of linkage maps, but also for gene tagging and QTL mapping. The information acquired is now being exploited to transfer different traits, including biotic stress resistances and improved quality traits, to important wheat varieties by means of marker-assisted selection (MAS) programs. In wheat, the most widely exploited techniques include AFLP and SSR markers which offer an almost unlimited supply of molecular traits for distinctive plant DNA fingerprinting and genotyping. A total of 38 durum wheat and 26 bread wheat DNA samples isolated from commercial varieties and experimental lines were investigated by fluorescent AFLP markers using five primer combinations previously selected on the basis of their ability of detecting polymorphisms. As many as 267 clearly detectable markers were scored, of which 59 (41.6%) and 73 (51.4%) proved to be polymorphic among varieties within and between species, respectively. Dice's (1945) genetic similarity (GS) estimates among the 64 pure lines were calculated in all possible pair-wise comparisons and the correspondent matrix was used for the construction of UPGMA dendrograms and the definition of centroids according to PCA analysis. Mean genetic similarity estimates within durum wheat and bread wheat were 92% and 89%, respectively. In each species, a few multi-locus genotypes showing almost full identity were found. Several species-specific and variety-specific DNA markers were also scored: the latter types will be cloned, sequenced and converted into easily detectable single-locus markers. On the whole, more than 68% of the total genetic variation found in wheat materials was explained by the first two principal coordinates. The observed number (na) and the effective number (ne) of alleles were equal to 1.416 and 1.163 in durum wheat and to 1.514 and 1.184 in bread wheat, respectively. Nei's (1973) genetic diversity (H) estimates over all genomic loci were also comparable for the two species (0.102 and 0.119, respectively). Linkage disequilibrium (LD) tests were performed for all pair-wise comparisons of marker alleles. The number of significant LD was 78 over 142 loci (0.78%) in durum wheat and 139 over 142 loci (1.42%) in bread wheat. Preliminary data suggest the finding of a few AFLP markers displaying highly significant linkage disequilibrium ($P<0.01$) with a number of wheat resistance genes, including yellow and brown rust, powdery mildew, Fusarium head blight and Septoria leaf spot diseases. The final aim is that of assembling a database of DNA polymorphisms for the durum and bread wheat germplasm. This

information is potentially useful not only for tracing single pure lines through genetic fingerprints, but also for planning experimental crosses between pure lines on the basis of their genetic distances.

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ISBN **88-900622-6-6**

Poster Abstract - C.07

DEVELOPMENT OF THREE GENETIC MAPS FOR THE DISSECTION OF THE GENETIC BASES OF AGRONOMICAL KEY TRAITS IN DURUM WHEAT

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linkage map, durum wheat, grain quality, stress tolerance, deseases

The main goal of durum wheat breeding consists of obtaining high yields with a good grain quality level. Protein content and gluten quality are the most important traits involved in determining pasta cooking value. Nevertheless a number of factors, due to extreme environmental conditions and to the presence of fungal pathogens heavily affect grain yield for both quantitative and qualitative aspects.

The use of genetic maps based on molecular markers could greatly enhance the manipulation of genetically complex traits such as grain quality, stress tolerance and disease resistance, supplying information on QTLs controlling these traits. Six cultivars characterized by different behaviour in terms of yield, grain quality, drought tolerance and resistance to fungal pathogens have been selected at the-Experimental Institute for Cereal Research, section of Foggia, and used as parental lines of three segregating populations: Creso x Trinakria, Ofanto x Cappelli and Cirillo x Neodur. Creso and Trinakria have been selected for different drought tolerance while Ofanto and Cappelli for high yields under favourable and unfavourable conditions. Cirillo and Neodur, as well as Ofanto and Cappelli, show a great difference in gluten index (higher in Cirillo and Ofanto than in Neodur and Cappelli), despite they are very similar for protein content, indicating that the different quality traits are related to gluten strength . Some varieties are also characterised by a different level of resistance to *Septoria Tritici*. Infact, Creso and Cappelli are clearly more resistant to this pathogen respect to Trinakria and Ofanto.

F7 seeds for about 110 lines are now available for each cross and about one hundred microsatellite and biochemical markers with know map position and covering all chromosomes have been found polymorphic between our cultivars. In particular, 86 markers have been found polymorphic between Creso and Trinakria (a average of 6 markers per chromosome), with a minimum of 3 markers on 4B and a maximum of 10 markers on 2A. A lower level of polymorphism characterises the other two parent couples: 67 markers have been individuated for Ofanto x Cappelli, for which a minimum of 4 markers are available for each chromosome, except 1A and 6A, and 54 for Cirillo x Neodur, with at least 3 positioned markers except for 6A, 5A and 4B chromosomes.

Finally, a number of common markers (19 common to the three crosses) will allow the construction of a consensus map to integrate mapping data from the three populations.

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ISBN **88-900622-6-6**

Poster Abstract - C.08

YIELD COMPONENTS AND ADAPTIVE TRAITS IN A SEGREGANT POPULATION OF DURUM WHEAT UNDER DROUGHT CONDITIONS

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drought, yield components, adaptive traits, QTL, durum wheat

In durum wheat the water scarcity is the primary constraint affecting grain production in the Mediterranean regions. In water stress conditions plant modifies his metabolic activity such as the accumulation of ABA, osmotic adjustment, ect. The risk of drought is highest during anthesis and the filling phase when a strong decrease of the grain yield can occur. In the past, morphological and yield components served as criterion for evaluating drought tolerance. The identification of QTLs for yield stability in water stress conditions is useful to develop marker assisted selection strategies.

A set of 120 recombinant inbred lines (RILs), obtained by single seed descent from the cross between the cv. Svevo and the cv. Ciccio, was cultivated by using the experimental design of split-plot, made up of two blocks with two different water levels: a rainfall one (stressed conditions) and an irrigated one (watered based on ET values). Plant adaptive traits (heading time, plant height, waxiness) and several yield components (grain yield, ear number, kernel number, 1000 kernel weight, grain yield per spike, hectolitre weight) were evaluated. Water stress conditions caused a significant decrease of grain yield. The statistical analysis shown that some yield components are more sensitive to drought in comparison to other. All yield components shown a low degree of heritability. Several molecular markers (microsatellites) polymorphic between the parental lines of the RIL population were identified. The recombinant inbred line population is being tested with microsatellites markers to identify QTLs involved in the genetic control of grain yield and its components under drought conditions.

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Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.09

CHARACTERIZATION OF DI- AND TRINUCLEOTIDE SSR MOTIFS IN WHEAT

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molecular markers, EST-SSR, polimorphic information content(PIC), wheat

Over the past decade microsatellites have attracted considerable attention of researchers. Microsatellite represent a valid alternative marker system, because of their abundance in plants genome, high level of polymorphism, and could be easily detected as PCR-based molecular markers on polyacrylamide or high resolution agarose gel. The advantages of SSRs include high information content, co-dominant inheritance, reproducibility and locus specificity. Recently a new source of SSR was represented by EST (expressed sequences tags). mRNA transcripts contain repeat motifs and actually the abundance of microsatellites in the expressed sequences tags of many species makes this markers very interesting because of a possible role in gene expression or function.

The aim of the present paper were to analyze EST-SSR variability in wheat and to investigate the relationships between type and number of repeat units and level of microsatellite polymorphism. 242 new EST-SSR available in public database (http//wheat.pw.usda.gov) were characterized in eight durum wheat cultivars (Svevo, Ciccio, Primadur, Duilio, Meridiano, Claudio, Latino, Messapia) three accession of *Triticum turgidum* var. *dicoccoides* (MG5323, MG4343, MG29896) and in the bread wheat cultivar Chinese Spring. Markers were opportunely chosen among di- and tri-nucleotide microsatellites in order to study relationships between number of repeat unit, type of motifs and level of markers polymorphisms. Of the 242 primer set tested, 80% produced one or two discrete PCR products. Markers based on di-nucleotide microsatellites were highly polymorphic in the 12 wheat genotype tested, approximately 53% of tri-nucleotide SSR were polymorphic compared to 73% of di-nucleotide SSR, with a average of 3.1 alleles for di-nucleotide SSR end 2.0 alleles for tri-nucleotide SSR. In conclusion 242 new EST-SSR were well-characterized and molecular analysis indicated dinucleotide SSR more powerful in the searching of polymorphisms.

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ISBN **88-900622-6-6**

Poster Abstract - C.10

MICRO-MORPHOLOGICAL TRAITS MEASURED BY IMAGE ANALYSIS, USEFUL FOR SELECTING DROUGHT TOLERANT WHEAT GENOTYPES

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micro-morphological traits, image analysis, wheat, drought

The need to realize appropriate yields in hot-dry environments is an old, but always actual, challenge of researchers, particularly for wheat, one of more important cereals economically.

Nowadays this challenge is carried out by all the scientific knowledge available in agronomic research.

The present study talks about micro-morphological characterization of anatomic structures by image analysis system connected to microscope. This system is able to realize all the measures of anatomic structures easily and it investigates their involvement in giving drought-tolerance feature to durum wheat.

Five Italian varieties of *Triticum durum* Desf. were analyzed. They were grown in three environments that were rainy different during vegetative cycle in the 1989-90 cropping season.

The vascular system was analyzed in the first and last internode (peduncle) and in flag leaf, in this leaf the stomatic apparatus was analyzed too, for a total of 49 parameters.

The obtained data were subjected to analysis of variance – one-way ANOVA (2 factors: 3 Location x 5 Varieties), mixed model; therefore the variation sources that resulted significant were undergone to a mean multi comparison test and the significance were tested with the Duncan test. All parameters were also submitted to correlation analysis (Pearson Correlation Analysis). Many parameters have showed interesting positive and negative associations, even with agronomic features such as production, 1000 seeds weight and hectoliter weight.

The varieties recorded values significantly different between them, both for their genetic characteristics and for their different reaction rule in the three different cultivation climatic environments.

This particular type of analysis, carried out only on micro-morphological traits of wheat plant, was able to characterize durum wheat varieties that adapt better to the difficult water stress conditions, ensuring satisfying yields.

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ISBN **88-900622-6-6**

Poster Abstract - C.11

A NEW BARLEY CONSENSUS FUNCTION MAP OF ABIOTIC-STRESS RELATED GENES

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function map, candidate genes, single nucleotide polymorphisms

The integration in an ideal genotype of favourable alleles at candidate genes having major effect on observed variability for tolerance to abiotic stresses will lead to improvement of barley productivity in stressful conditions. In order to study in an unique genetic system drought and cold stresses, a new barley consensus function map has been developed, based on three well characterized mapping populations: 'Nure' x 'Tremois' (NxT), 'Steptoe' x 'Morex' (SxM) and 'Proctor' x 'Nudinka' (PxN).

The candidate-gene (CG) strategy was here applied to find putative candidates for barley abiotic-stress tolerance: barley CGs were selected from literature and GenBank database screening on the basis of their involvement in abiotic-stress response. Greater importance was here given to transcription factors and regulatory genes rather than to structural genes, because a transcription factor usually can regulate the expression of several downstream stress-responsive genes.

Candidates were screened on the six parental genotypes using Single Strand Conformation Polymorphism (SSCP) technique, to search for Single Nucleotide Polymorphisms (SNPs) and short INsertion/DELetions (INDELs). Depending on the polymorphism type, new CAPS, ARMS-PCR and SSCP markers were developed to map CGs on the NxT, PxN and SxM populations. Based on the presence of markers in common to the three linkage maps, segregation data were analyzed with anappropriate software to create the initial barley consensus function map.

The inferred position on the molecular consensus map of previously reported QTLs for barley frost- and drought-tolerance revealed, among the functional candidate genes mapped, some interesting positional candidates. These results represent a good example of the application of the candidate-gene approach for the dissection of a complex phenotypes such as the tolerance to abiotic stresses. Complementary validation experiments are being conducted to confirm the actual involvement of co-segregating CGs in the traits variation.

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ISBN **88-900622-6-6**

Poster Abstract - C.12

ISOLATION OF CANDIDATE GENES INVOLVED IN BARLEY DEVELOPMENT

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candidate gene, synteny, barley, SNP, development

A synteny approach between the genomes of rice and barley was adopted to individuate candidate genes (CGs) for the barley developmental mutants previously mapped by our group. Map positions were used to select sequenced RFLPs linked with the mutant loci. *In silico* mapping of these markers by BLAST searches against the rice genome sequence allowed the identification of syntenic chromosomal regions in rice. CGs of particular interest include the rice ortholog of maize *Liguleless1* (*Lg1*), which represents a candidate for the barley *liguleless (lig)* locus, and the rice *FRIZZY PANICLE (FZP)* gene, which represents a candidate for the barley *branched1* (*brc1*) locus.

A fragment of the barley *Lg1* gene was isolated by PCR with degenerate primers and mapped *via* SNP mapping on chromosome 2H, in the region hosting the *lig* locus, providing preliminary evidence of the correlation between CG and mutant phenotype. Cloning of the entire gene sequence and cosegregation analysis with the mutant phenotype are underway.

The *brc1* locus (previously *brc*-5, Franckowiak and Lundqvist 2002) was mapped on barley chromosome 2H, sublinkage group 17 (Castiglioni et al. 1998). A syntenic chromosomal region was defined on rice chromosome 7. Annotation of this region resulted in the identification of *FRIZZY PANICLE (FZP)*, a rice gene involved in inflorescence architecture and orthologous to maize *Branched Silkless1* (*BD1*). The barley orthologue was isolated and is being SNP-mapped on the high-density molecular map developed in our lab, to corroborate the correlation between the locus and the mutant phenotype.

A strong CG was also found for the *calcaroides* b19 and *calcaroides* C15 loci located on barley chromosome 5H. The rice syntenous region for these loci hosts a predicted gene exhibiting high sequence similarity to *rough sheath2*, a MYB gene implicated in the negative regulation of *knox* activity in maize (Timmermans et al. 1999; Tsiantis et al. 1999). The barley *rough sheat2* (*Brs2*) homologue was isolated in our group and its expression characterized by *in situ* hybridization on barley vegetative apexes and immature inflorescences. The gene shows a strong expression in a ring-shaped domain at the lemma basis. Two experimental evidences are contrasting with a possibile functional role of *Brs2* in the *calcaroides* phenotype: a. differences in gene expression between the wild type and *calcaroides* mutant were not evident from reverse transcription experiments; b. *Brs2* was mapped on chromosome 7H, in proximity to the *short awn* (*lks*2) locus. Cosegregation between the MYB gene and the *lks*2 locus is under investigation.

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Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.13

MARKER-ASSISTED SELECTION FOR *MLO*-MEDIATED POWDERY MILDEW RESISTANCE IN BARLEY

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barley, powdery mildew resistance, mlo, SNPs, marker-assisted selection

Recessive *mlo* alleles of the barley *Mlo* gene confer resistance to almost all known isolates of the barley powdery mildew fungal pathogen. We sought to generate PCR markers suitable for selection of *mlo* in breeding populations. Towards this end, a search for single nucleotide polymorphisms (SNPs) and insertion/deletions (InDels) was carried out in three chromosomal regions adjacent to the *Mlo* gene. Analysis of sequences from four *Mlo*- and from three *mlo*-containing cultivars allowed the detection of eight SNPs and one InDel. PCR-based markers were subsequently developed for typing the SNPs. Molecular markers were analyzed within 36 *Mlo* and 25 *mlo* barley cultivars. At least two molecular markers could distinguish each of 28 *Mlo* barley cultivars from all of the *mlo* barley cultivars. In progeny of crosses between these resistant and susceptible lines, the identified markers could therefore be used to select *mlo* genotypes. To verify the utility of the markers, they were used to analyse *mlo*-segregating F_2 families derived from two different crosses. The marker alleles correctly predicted the *Mlo* locus genotypes in both crosses, indicating that the markers could be valuable tools for selection of *mlo*-mediated powdery mildew resistance in barley

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.14

IDENTIFICATION AND MAPPING OF RESISTANCE LOCI TO *PYRENOPHORA* SPP DERIVED FROM *HORDEUM SPONTANEUM*

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barley, Pyrenophora teres, P. graminea, mapping

Net blotch and leaf stripe caused by the fungi *Pyrenophora teres* Drechsler and *P. graminea* Ito & Kuribay respectively, represent serious threats to grain yield in barley (*Hordeum vulgare L.*). The wild progenitor of cultivated barley, *H. spontaneum* L. represents a useful source of resistance to various biotic stresses for the development of new resistant varieties. The evaluation of a *H. spontaneum* accession 41-1 to *P.graminea* and to *P. teres* demonstrated, respectively, full resistance and partial resistance to these disease. A medium-density, molecular marker map derived from a segreganting population of recombinant inbred lines (RILs) obtained from the cross between *Hordeum spontaneum* 41-1 x 'Arta' (susceptible) was available. The inoculation of one hundred and ninety four RILs with the two pathogens lead to the identification and mapping of QTLs involved in the resistances. PCR-based molecular markers linked to these loci were also developed in order to improve the mapping; these marker can also represent useful tools for the introgression of these resistant loci in susceptible barley cultivars by molecular marked assisted selection.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.15

TOWARDS THE FUNCTIONAL CHARACTERIZATION OF A RICE *WRKY* TRANSCRIPTION FACTOR

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transcription factor, insertion mutant, GUS expression, gene family

WRKY genes belong to a heterogeneous family of transcription factors sharing a 60 aa conserved consensus sequence that provide DNA-binding properties to a target sequence (T)(T)TGAC(C/T). Although one copy of this gene is present in some unicellular organisms such as *Giardia lamblia*, *Trypanosoma brucei* and *Dictyostelium discoideum*, indicating their very ancient origin, they were apparently lost in fungi and animals, but underwent to extensive duplication events in the plant kingdom which produced 72 members in Arabidopsis and 107 members in rice. *WRKY* genes seem to be involved in several biological processes such as responses to the abiotic stresses of wounding, the combination of drought and heat and cold. It is also evident that some members of the family may play important regulatory roles in morphogenesis of trichomes, embryos, senescence, dormancy and metabolic pathways. Many *WRKY* genes are involved in response to biotic stresses. In an effort to verify the involvement of *WRKY* genes of rice in plant response to pathogens, we screened 15 lines containing insertion in *WRKY* genes, inoculated with host and non-host strains of *Magnaporthe grisea* (isolates BR29, BR32 and FR13). No phenotype was detected in such lines but a T-DNA insertion event in the promoter of *OsWRKY55* showed a tissue-specific Gus expression in roots, vascular tissues and in the cotyledon of a 10-days old seedling. According to our phylogenetic analysis, this gene belongs to a subgroup of Monocot specific *WRKY* genes. A more detailed analysis is currently ongoing to characterize the expression pattern of this *WRKY* gene and possibly to identify a phenotype correlating with the insertion.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.16

ISOLATION AND PRELIMINARY CHARACTERIZATION OF A NEW BRACHYTIC MAIZE MUTANT

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maize, mutant, brachytic, P-glycoprotein

Plants with short stature have had a big impact on world agriculture. This idea is exemplified by success of the green revolution, which was made possible by the use of dwarf varieties of wheat and rice. Brachytic/dwarf varieties have not been exploited commercially in maize, partly because of the excessively severe nature of the original mutants alleles. However similar mutations have been used extensively in sorghum production since the 1950s.

A maize brachytic mutant of agronomic potential is the recessive *brachytic2* (*br2*) mutation, which results in the shortening of lower stalk internodes, no other plant organs, including mesocotyl, coleoptile, leaves, ear, and tassel are affected in size or growth.

The brachytic maize mutant, named *brachytic-23** (*br*-23*), described in this work was originally observed in a selfed B73 family. This suggests that the mutation occurred spontaneously in the previous generation. This mutant has a short stature and compact lower stalk internodes compared to wild type control. The genetic analysis indicated that *br*-23* was inherited as a monogenic recessive trait.

To ascertain the relationship of the new brachytic mutation to previously isolated *br1*, *br2* and *br3* mutations, we crossed these mutants inter se in all pair wise combinations to assay their pattern of complementation. The results obtained show that *br*-23* mutant fails to complement *br2* suggesting an allelic relationship. To corroborate this data, the map position for the new brachytic mutation was achieved by the analysis of simple sequence repeat (SSR) marker-distribution in a F2 segregating population consisting of about 150 plants obtained from the selfing B73 *br*-23/br*-23* x A636 plants. Mapping data confirm the position of *br*-23* in the same genomic region of the *br2* allele previously described. On the whole, these data indicate that *br*-23* mutant bears a lesion in *Br2* gene. According to the guidelines indicated by the Maize-GDB we renamed the new mutation by the provisional designation *br2-23*. Recently, *br2* was cloned by transposon tagging with *Mu* element by Multani et al., in 2003 and it encodes a putative protein similar to adenosine triphosphate (ATP)-binding cassette transporters of the multidrug resistant (MDR) class of P-glycoproteins (PGPs) involved in polar movement of auxins.

Details of further molecular, genetic and histological characterization on this mutant will be presented.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.17

IN SITU CONSERVATION OF MAIZE LANDRACES IN EUROPE

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genetic resources, landraces, Zea mays, molecular diversity

Maize *landraces* are still cultivated in different parts of Europe, probably due to their specific adaptations to traditional farming systems and their association with the production of traditional foods (e.g. polenta in Italy). In contrast to both conventional and genetically modified (GM) maize, this dynamic conservation of landraces implies that seeds are produced on the farms year after year. Within the EC Sixth Framework Programme, the ongoing SIGMEA (Sustainable Introduction of Genetically Modified Crops into European Agriculture) project (http://sigmea.dyndns.org/) is designed to identify the distribution of the cultivation of maize landraces in Europe and to determine the impact of past gene introgression from hybrid varieties into landraces, in order to define the potential impact of the introduction of GM organisms into European agriculture. Here, we present two study cases, of which one concerns *in situ* conservation of maize landraces in the Maramures Region of Romania, while the second is comparing recent (2000) and older (1950) accessions of maize landraces collected from the Marche Region in Italy. Following a survey in 2002 by the Suceava genebank team in the Maramures area, 49 landraces of maize were identified and collected from 15 villages. Improved maize cultivars have been introduced into the area, which belong to the dentiformis variety, although old types are regarded as having better gastronomic qualities and are preferred for their nutrition.

The analysis of genetic diversity of maize *landraces* from Marche, Italy, was conducted using two samples of maize *landraces*, one collected in 1950 by the Istituto per la Cerealicoltura di Bergamo (47 accessions, 90 genotypes) and the other in 2000 by DiSA-Università Politecnica delle Marche (20 accessions, 77 genotypes). The study also includes one sample of traditional *landraces* characteristic of North Italy (7 accessions, 14 genotypes) and a sample of modern hybrid varieties of 6 flint and 2 dent corns. All of the materials were analysed using 6 AFLP primer combinations and 6 SSR *loci*. A subset (73) of accessions were also compared in a field trial and evaluated for phenotypic traits.

The molecular analysis indicates that the two samples of maize *landraces* from Marche collected the time period that spans about 50 years are highly inter-related, and they are clearly different from both the flint and dent corn hybrid varieties. On the other hand, the phenotypic evaluation shows that the recent collections of *landraces* are intermediate between the old collections and the modern hybrid varieties. Overall, our results indicate that gene flow between *landraces* and hybrid varieties has not affected the genetic structure of maize *landraces* from Marche, even if the results of the phenotypic traits suggest the occurrence of moderate introgression from hybrid varieties as well as the occurrence of farmer selection for useful agronomic variants.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.18

CO-LOCATION OF QTLs FOR SILENT GENETIC VARIATION AND FOR HETEROSIS IN MAIZE

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heterosis, Hsp90, Quantitative Trait Loci(QTLs), Zea mays L.

Heat-shock protein 90 (Hsp90) is an abundant cytosolic protein that contributes to homeostasis under physiological and stress conditions (Buchner, 1995). Studies conducted on *Drosophila* and *Arabidopsis* showed that the use of Hsp90 inhibitors, such as geldanamycin (GDA), can reveal silent genetic variation (Queitsch *et al*, 2002) involved in adaptation to peculiar environmental conditions. Moreover, Hsp90 proved to chaperone the signalling proteins that control plant growth and development. Therefore, it appears that manipulating Hsp90's buffering capacity offers a tool for harnessing cryptic genetic variation and for elucidating the interplay between genotype and environment in the determination of phenotype (Sangster *et al*., 2004).

It has been proposed that the silent variability can be involved in hybrid vigour. Since F_1 hybrids are generally characterized by higher stability than their parents, challenging Hsp90 in genotypes with different levels of heterozygosity could reveal hidden genetic buffers, thus providing useful clues on the genetic control of heterosis. The exploitation of heterosis has been considered one of the most revolutionary advancements in plant improvement, but its genetic basis is not yet completely understood. In a previous study (Frascaroli *et al.*, 2004), the genetic control of heterosis in maize (*Zea mays* L.) has been investigated combining both classical and molecular methods. That study allowed the identification of important QTLs determining heterosis.

The aim of this work was to identify QTLs controlling the response to chemical compounds inactivating Hsp90 and to verify their possible co-location with the QTLs controlling heterosis.

The plant material utilized in this work derived from a mapping population of 142 RILs of the cross B73 x H99 already characterized for more than 200 SSRs and AFLPs markers. Each RIL was crossed to both parental inbreds to obtain two pseudo-backcross (YBC) families per RIL. All YBC families were tested both in absence and in presence of the Hsp90 inhibitor (GDA). Seeds were sterilized and left overnight in a solution containing distilled water and DMSO (control) or DMSO plus GDA (treated). Soaked seeds were transferred to Petri dishes then incubated at 20 °C for 21 days. The response to the treatment was evaluated as difference between control and treated for shoot length, primary root length and quality of root development (as a score of root curling, twining and abnormal hair production).

QTL analysis was performed on YBC data, by adopting a model allowing the estimation of additive and dominance effects. Several QTLs were detected for reaction to GDA in all traits. Most of them (on chromosome 2, 3, 6, 8 and 10) co-located with major QTLs controlling heterosis found in our previous work for grain yield and other agronomic traits. These preliminary findings can stimulate interesting new hypotheses regarding the explanation of the biochemical basis of heterosis.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.19

CIS-ACTING REGULATORY VARIATION AFFECTING MAIZE GENE EXPRESSION

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cis-variation, allelic expression, maize

Mutations in *cis*-regulatory DNA sequences, which affect gene expression levels, have been proposed to influence quantitative variation, disease susceptibility, and to be a primary substrate for the evolution of the species.

Our recent studies of allele-specific expression in a random set of non-imprinted maize (*Zea mays*) genes in F1 hybrids demonstrated that more than 70% of the genes show allelic differences in expression of at least 1.5 fold due to *cis*-regulatory variation and that these differences are tissue specific. We further examined allele-specific expression under different types of abiotic stress. Stress induced significant imbalance in expression ratios for the alleles that initially showed no disparity in expression, and it enhanced alteration in the relative expression ratios for those that originally showed differential expression of the two alleles.

Maize genome is known to harbor especially high nucleotide diversity within single copy regions, including genes, and lack of colinearity in intergenic regions due to the presence of different LTR-transposons which is making it a unique example of within species diversity. To investigate the potential for *cis*-acting variability in genes analyzed we sequenced the 5' flanking regions and ORFs of 5 of them in a sample of 17 inbred lines and assessed nucleotide diversity and linkage disequilibrium. In attempt at identifying *cis*-acting elements responsible for the differences in allelic expression, upstream regions were screened for putative binding sites and phylogenetic footprinting was performed. We also estimated allelic expression imbalance in genes within fully sequenced genomic regions from both alleles to assess the effects of the lack of colinearity in intergenic regions.

Our findings suggest that the *cis*-regulatory variation is a highly common phenomenon in maize and may provide a possible molecular explanation of the heterosis phenomenon. The heterozygous state found in hybrids for many genes may represent a buffering mechanism to improve stress tolerance and more in general to ensure optimal gene expression in a wide variety of environmental conditions.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.20

HELITRON-LIKE ELEMENTS MEDIATE EXTENSIVE GENE DUPLICATIONS AND EXON SHUFFLING IN MAIZE

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helitron, DNA transposon, maize, polymorphism

Widespread occurrence of DNA sequence non-colinearity among maize inbreds has recently been reported. This has been attributed to recent LTR-retrotransposon insertions and to the presence/absence of genes or gene fragments. The molecular mechanism responsible for the gene content differences has not been elucidated, even though the evidence points to insertion rather than deletion events. Here we report a whole-genome comparison of gene content in allelic maize BAC contigs from two maize inbreds and show that gene presence/absence polymorphisms are very frequent in the maize genome. The allelic differences may involve as many as 10000 genes when genomes of two common maize inbreds are compared. The termini of eight out of nine of the genic insertions we examined in detail share the structural hallmarks of the rolling-circle transposon class named helitron. Closely related genic insertions equipped with helitron termini are found in multiple genomic locations, suggesting they are non-autonomous transposons. They usually contain multiple gene-derived fragments in the same orientation. These sequences can be transcribed across segments derived from different genes, suggesting their possible participation in exon shuffling and evolution of novel protein functions. We also identified putative autonomous helitron elements in maize genomic sequences. The expression of helitron ORF coding for a protein with helicase and replication initiator activities was confirmed by RT-PCR on mRNA from seedlings in 18 inbred lines. Analysis of expressed sequences indicates that more than one helitron subfamily is expressed and that different lines express different subfamilies.

Thanks to their ability to produce a very diverse set of non-autonomous elements helitrons are responsible for the frequent duplicative insertion of gene segments into new locations in maize and for a profound reshuffling of the maize genome, leading to an unprecedented gene content diversity within a species as well as between maize and related species.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.21

CHARACTERIZATION OF THE *Ra1* MAIZE GENE INVOLVED IN INFLORESCENCE ARCHITECTURE

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maize, inflorescence, ra1 mutant, zinc finger domain

Plant and floral architecture has an enormous impact on yield, either by altering the numbers of fruits and seeds produced by the inflorescence, or by making plants more compact allowing them to be grown under stringent conditions. In maize, increasing the number of branches in the tassel increases pollen yield, which influences overall yield as well as F1 production.

Several mutations have been described that affect the development of the maize inflorescence, but only *ramosa1* (*ra1*) causes a change in a specific branching pattern of the inflorescence without a concomitant loss of any tissue types.

Ra1 gene was cloned and preliminarily characterized by Martienssen et al., it encodes a small zinc finger transcription factor (patent number: WO 01/90343 A2).

In the present work, we have characterized a new mutation of *Ra1* gene, named *ramosa1-154* (*ra1-154*), isolated in the progeny of a selfed B73 inbred line plant. This spontaneously arising ramosa maize mutant shows over-branching in the male and female inflorescences. This over branching causes a net increase of the number of spikelets in the tassel of about 50% respect to wild type.

Also heterozygous *Ra1/ra1-154* plants exhibited a consistent increase in number of branches and spikelets in the tassel compared to wild type, this might indicate that one single-functional gene copy is not sufficient to warrant the wild phenotype. This data indicate that the number of tassel branches is closely linked to RA1 level just as, most probably, the different average number of tassel branches present in several inbred lines might be due to different expression level of different *Ra1* alleles.

We cloned and sequenced *Ra1* gene in *ra1-154* mutant and wild type. The comparison of the sequences showed the presence of AAG deletion (position +157) in the coding region in *ra1-154* mutant. This small deletion causes the loss of the K residue at the position 53 in the predicted putative zinc finger domain of RA1 protein.

This is the first evidence of single amino acid deletion in the zinc finger domain that knocks out the function of the RA1 protein.

This result strongly suggests that the RA1 protein functions by acting as a DNA-binding protein, likely involved in transcriptional regulation and in particular, the presence of ERF-associated amphiphilic repression (EAR) motif in RA1 protein sequence supports the hypothesis that *Ra1* gene might acts as repressor of genes involved in the inflorescence branch meristems.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.22

TOWARDS THE TILLING IN TOMATO: CONSTRUCTION OF A MUTANT PHENOTYPE DATABASE

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TILLING, mutagenesis, tomato, phenotype, database

EthylMethaneSulfonate (EMS) mutagenesis is a standard technique used to induce point mutations in DNA and to create a genetic background for exploring gene function and for isolating new genotypes with traits of interest.

In order to create new genetic resources ("core collections"), we developed two mutant populations by treating the *Red Setter* tomato seeds with two different EMS concentration (0.7% and 0.1%). A total of 18000 M2 plants, grown in greenhouse soil and open field, are being phenotypically analyzed and their data and images organized in a database.

0.7% and 1% EMS tomato mutants are being assigned to categories such as: 1) number and colour of cotyledons, 2) shape and size of tomato plant, 3) leaf morphology and colour, 4) flower morphology and colour, 5) flowering time, 6) colour, size, morphology and fertility of the fruits.

Most of the mutant phenotypes result to fall in more than one category. A high percentage of the mutations affect: 1) tomato leaf morphology and 2) size and shape of the plant while a low percentage of mutations have been found to affect flower structure and colour.

The phenotyped populations will be utilized for reverse genetic analysis (TILLING) and for the identification of tomato lines carrying agronomic and nutritional traits of interest.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.23

BIOTIC AND ABIOTIC STRESS RESISTANCE OF (ANEUPLOID) PENTAPLOID *SOLANUM COMMERSONII – S. TUBEROSUM* HYBRIDS

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sexual hybrydization, ploidy bridges, chromosome number

The potato (*Solanum tuberosum* L.) is the fourth most important food crop worldwide after wheat, maize and rice. Even if it is characterized by a narrow genetic basis, there is a large number of tuber-bearing *Solanum* species which represent a source of noteworthy traits. Among diploid *Solanum* species, *S. commersonii* possesses several desirable traits, including high specific gravity of tubers and resistance to biotic and abiotic stresses. *S. commersonii* is sexually isolated from *S. tuberosum* due to post-zygotic barriers. For this reason, alternative approaches to the "analytic breeding" have been developed, such as a "ploidy bridge" strategy based on the production of F_1 triploid and BC_1 pentaploid bridges. Used in this study were 30 BC_1 *S. commersonii – S. tuberosum* pentaploid bridges. They have been evaluated for chromosome number and resistance to *Ralstonia solanacearum, Phytophtora infestans, Erwinia carotovora,* and low temperatures. Cytological analysis showed a large variability in terms of chromosome number (2n=52÷67), probably due to the mechanism of restitution of the nucleus in second meiotic division that allowed the production of 2n eggs in the triploid parents. Also, through laboratory tests, it has been possible to identify genotypes with positive traits. Hybrids with resistance to *Ralstonia* and *Erwinia* were selected. As for *Phytophtora,* all genotypes tested showed low level of resistance. Results from experiments aimed at evaluating the resistance to low temperatures provided evidence that acclimation capacity was transferred from wild *S. commersonii* to BC_1 hybrids. No significant correlations were found between chromosome number, resistance traits and other phenotypic characteristics. This indicated that the aneuploid chromosome complement did not affect traits evaluated.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.24

CHARACTERIZATION OF PROGENIES FROM INTER-PLOIDY, INTER-EBN CROSSES IN *SOLANUM*

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potato, aneuplod, haploid, Endosperm Balance Number (EBN)

A ploidy bridge strategy was followed to overcome isolation barriers due to EBN differences between wild *Solanum commersonii* (*2n=2x=24*, 1EBN) and S. *tuberosum* haploids (*2n=2x=24*, 2EBN). Genetic materials generated was interesting not only for variety development but also for basic research. In fact, the ploidy levels produced (triploid, pentaploid, aneuploid) are hardly available and they can provide important information on evolution, reproduction and EBN in potato and other polysomic polyploids. In this study, we present the cytological and molecular characterization of progenies obtained for *3x*(2EBN) *x 2x*(2EBN), *5x*(4EBN) *x 2x*(2EBN) and *4x*(4EBN) *x 2x*(2EBN) crosses. Cytological analysis of *3x*(2EBN) *x 2x*(2EBN) progenies provided evidence that chromosome number ranged from *2n*=29 to *2n*=36, and that trisomics were not obtained. It is possible to hypothesize that in these crosses the EBN incompatibility system favoured gametes of triploid parent with a high number of extra-chromosomes. This would generate a 2:1 maternal to paternal EBN ratio in the hybrid endosperm, that is the necessary condition for normal endosperm development. Cytological analysis of progenies obtained from *5x*(4EBN) *x 2x*(2EBN) and *4x*(4EBN) *x 2x*(2EBN) crosses underlined the production of haploid, euploid and aneuploid genotypes. Meiotic restitution mechanisms and exceptions to the EBN model are probably the basis of these results, as also supported by molecular analysis with ISSR markers. Our findings confirmed the importance of the EBN system in predicting success of inter-ploidy, inter-EBN crosses in potato and provided genetic material useful for interspecific gene flow.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.25

DEVELOPMENT OF MOLECULAR MARKERS LINKED TO THE RESISTANCE TOWARDS *FUSARIUM OXYSPORUM* F. SP. *MELONIS* RACE 1,2W IN MELON

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resistance gene, Fusarium wilt, AFLP, Cucumis melo L.

Fusarium oxysporum f.sp. *melonis* (FOM) causes serious economic losses for melon (*Cucumis melo* L.). Two dominant resistance genes have been identified, *Fom-1* and *Fom-2*, which confer high levels of resistance to races 0 and 2 and races 0 and 1, respectively. However, FOM race 1,2 overcomes these resistance genes. Race 1,2 was further subdivided into two pathotypes, one that causes wilting (FOM 1,2w) and one that causes yellowing (FOM 1,2y). No genes have been identified in melon which can provide resistance to either race 1,2y or race 1,2w. However, a partial resistance to FOM race 1,2, which is under polygenic control, has been found in some Far East accessions. Recently, the identification of nine QTLs involved in FOM 1,2 resistance in melon was reported for the first time. The aim of the present work was to develop molecular markers linked to resistance towards *Fusarium oxysporum* f.sp. *melonis* race 1,2w in the DH melon lines to be used in plant breeding. For the molecular analysis, DNA was extracted from 192 F2 plants of the two segregant populations developed by hybridization between the resistant genotype Nad-1 (N) and the susceptible genotype Charentais-T (C) using reciprocal crossing.

A modified bulk segregant analysis was carried out using 75 AFLP primer combinations to screen the parents and each of three bulks of 7 resistant plants, assuming that all the F2 plants that originated the F3 families showing 100% resistance were homozygous for the resistant genes. Forty out of 75 AFLP primer combinations were chosen on the basis of fragments present in the susceptible genotype and absent in the bulks and the resistant parent, and they were used to test each of the individual F2 resistant plants of the three bulks previously described. Ten out of 40 AFLP primer combinations were than selected to score the parental genotypes and the 60 F2 individual plants of the two segregant populations. Significant linkage association ($P<0.01$) of the developed AFLPs with the resistance to race 1,2w of *Fusarium oxysporum* f.sp. *melonis* was found for four fragments.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.26

EFFECTIVENESS OF PHENOTYPIC SELECTION OF "BROCCOLO FIOLARO" (*BRASSICA OLERACEA*) ASSESSED BY MOLECULAR MARKERS

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genetic diversity, SSR markers, cultivar protection, phenotypical evaluation

"Broccolo fiolaro" is a typical vegetable produced in a restricted hill country area around Creazzo, Vicenza-Italy. The cultivation of this vegetable dates back to some centuries ago, but at present very few farms are still involved in the production. Broccolo Fiolaro is a botanical variety of cabbage highly valued for its agromonic and organoleptic features.

Four "Broccolo Fiolaro" lines were derived by phenotypic selection carried out on 200 plants growing into the field far away each others. During the winter season the tuft size, leaf size and shape were recorded. At the spring time the earliness in stem development and the number of branches were observed. For each population, the most uniform (15-20) plants were selected and left to free pollination.

Their progeny was analyzed by molecular markers (SSR and AFLP) and compared with 8 cabbage populations in order to evaluate the genetic structure and similarity among and within populations. Rapa "Chiampo" (Brassica rapa) was considered as an outgroup. Up to now 12 SSR primer pairs were used and the preliminary results point out low genetic variability within the four "Broccolo Fiolaro" selections. On the contrary other Broccolo types such as "Bassano", "Riccio di Sarno" and "Liscio di Napoli" as well as Brussel cabbage and the outgroup show allele polymorphism at the loci analyzed and high genetic variability.

It seems that phenotypic selection was effective in the reduction of genetic variability within the selections of Fiolaro type.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.27

GENETIC DIVERGENCE ANALYSIS IN EGGPLANT (*SOLANUM MELONGENA*) AND ALLIED SPECIES

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germplasm, genetic diversity, Solanum spp*., multivariate analyses*

Genetic divergence in 98 accessions of *Solanum melongena* (55) and his allied species *S. aethiopicum* (27) and *S. macrocarpon* (16) for 16 morpho-agronomic and fruit traits revealed the existence of considerable diversity. Such collections were grown in the field during the five years EU-EGGNET project for characterization and seed multiplication. Diversity has been observed between the different species as well as within the species. Frequency distributions for fruit pedicel length, bitter flavour, browning, peelability, and cooking test were determined. Beside the qualitative descriptors 11 quantitative descriptors were described. The relationships among them were analysed by the Principal Component Analysis to summarize the data and reduce the number of variables for clustering. Plant height, flowering time, flower/inflorescence, fruit length and fruit acidity contributed most towards total divergence. Cluster analysis conducted separately for each species in relation to the genetic status of accession (sub-species, botanical group, cultivar, landrace, population) grouped the accessions into 3 distinct and significant clusters. No relationship was found between genetic divergence and genetic status of sample. In addition, relevant fruit discrete descriptors were used as classification variable to find out whether some of them correspond to certain morpho-agronomic properties. The genotypes included in the diverse clusters could be used as promising parents for hybridisation to obtain high heterotic response and thus better segregants in eggplant.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.28

MOLECULAR AND ISOENZIMATIC CHARACTERIZATION OF A DIHAPLOID POPULATION DERIVED FROM ANTHER CULTURE OF THE SOMATIC HYBRID *S. MELONGENA + S. AETHIOPICUM* gr. *GILO*

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Solanum melongena, Solanum aethiopicum, somatic hybridization, Fusarium oxysporum f.sp. melongenae, ISSR

Solanum aethiopicum gr. *gilo* is a close wild relative species of *S. melongena* which show resistance to *Fusarium oxysporum* f. sp. *melongenae* and to some strains of *Ralstonia solanacearum,* two soil borne and very destructive diseases of eggplant. In order to access wild germplasm by overcoming the sexual barriers between this species and eggplant, a tetraploid somatic hybrid [*S. aethiopicum* (+) *S. melongena* cv Dourga] was obtained by electrofusion of protoplasts. Five fully resistant somatic hybrids, grown in glasshouse, were used as anther donors to obtain diploid androgenetic plants that potentially could be backcrossed with the cultivated eggplant (Rizza et al, 2002). The resulting dihaploid progenies (DH) were submitted to bio-morphological characterization and the range of variation of the measured traits resulted distributed within the extreme values of the two fusion parents and around that one of the somatic hybrids. Moreover, new combination of traits were displayed in single dihaploid plants and the resistance to *Fusarium* segregated in the DH progeny. Qualitative and quantitative evidences suggest that genetic recombination between the genome of the two parental species occurred during meiosis and it is phenotypically expressed in the DH plants.

DH population was also subjected to biochemical characterization, testing 7 different isozyme systems, and to molecular study, through the analysis of data from amplification with 32 polymorphic ISSR primers.

Isozyme and ISSR molecular analyses demonstrate the hybrid condition of the fusion products and evidenced that recombination and segregation occurred in the dihaploid progenies. However, segregation ratio in the DH population was consistently different with respect to the expected values.

This result confirm that genetic recombination partially took place between homeologus chromosomes of the two species. The somatic hybrids, therefore, may be considered as segmental allopoliploid.

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Rizza, F., G. Mennella, C. Collonnier, D. Sihachakr, V. Kashyap, M.V. Rajam, M. Prestera', G.L. Rotino, 2002 – Androgenetic dihaploids from somatic hybrids between *Solanum melongena* and *S. aethiopicum*

group *gilo* as a source of resistance to *Fusarium oxysporum* f.sp. *melongenae*. Plant Cell Report 20: 1022-1032.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.29

CHARACTERIZATION OF PLANTS OBTAINED FROM "BACKFUSION" BETWEEN SOMATIC HYBRID-DERIVED DIHAPLOIDS AND EGGPLANT

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Solanum melongena, Solanum aethiopicum, somatic hybridization, Fusarium oxysporum f.sp. melongenae, ISSR.

One of the major limitations for incorporation of somatic hybridization into applied breeding of eggplant is generally represented by the tetraploid level of the somatic hybrids obtained. Somatic hybrids of eggplant with different allied species have been produced (e.g. *S. aethiopicum, S. integrifolium, S. sisymbrifolium, S. torvum*). Reduction of the ploidy level to the diploid status may facilitate the backcross with the cultivated diploid eggplant. Dihaploid androgenic plants have been obtained through anther culture of the somatic hybrid between eggplant and the allied specie *S. aethiopicum.* Morphological, biochemical, molecular and biological features of the androgenetic dihaploids plants demonstrate that genetic recombination between the genome of the two species took place in the somatic hybrids (Rizza et al, 2002; Toppino et al, 2005). Although this finding open up the possibility to introgress useful traits (e.g. resistance to *Fusarium oxysporum* f.sp. *melongenae* and *Ralstonia solanacearum*) into the eggplant gene pool, the dramatic reduction of the fertility of dihaploids hampers their practical exploitation in breeding program by sexual crosses. In order to overcome such hindrance, the dihaploids were subjected to protoplast "backfusion" with eggplant. This method was used to obtain tetraploid somatic hybrids in which the genome of eggplant would be prevalent and, consequently, the dihaploids extracted by anther culture should be more fertile and suitable for backcrossing.

Leaf protoplasts of *in vitro*-grown plantlets from two different dihaploids resistant to *Fusarium oxysporum* and from the eggplant parental cv. Dourga were electrofused. Data about phenotypic (floral and fruit characteristics), biological (pollen viability), ploidy level and ISSR molecular characterization of the regenerated backfused somatic plants are presented and discussed.

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Rizza, F., G. Mennella, C. Collonnier, D. Sihachakr, V. Kashyap, M.V. Rajam, M. Prestera', G.L. Rotino, 2002 – Androgenetic dihaploids from somatic hybrids between *Solanum melongena* and *S. aethiopicum* group *gilo* as a source of resistance to *Fusarium oxysporum* f.sp. *melongenae.* Plant Cell Report 20: 1022-1032.

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Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.30

PROGRESS IN BREEDING FOR INNOVATIVE CHARACTERS IN MEDITERRANEAN GRAIN LEGUMES

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Faba bean, lentil, blue lupine, breeding, innovative characters

In 1993 the Authors started a large programme of collection of germplasm and of breeding for introducing several innovative characters into locally adapted varieties and populations of several Mediterranean grain legume species, for both human and animal utilization: faba bean, lentil, chickpea, white lupine, blue lupine, grasspea. In the present contribution are reported up to date results obtained in faba bean, lentil and blue lupine.

In faba bean the important characters introduced into *minor, equina* and *maior* types were: ”close flower” and “self fertility”, inducing strict autogamy and normal productivity; “pure white flowers” and “white hylum”, associated with absence of tannins in the plant and seeds; different seed sizes and *testa* seed colours; high fertility and productivity. Strict autogamy could be considered a new domestication character of high value for breeding and seed production of the species which normally has a large amount of natural cross pollination.

In lentils the characters introduced were: “white testa colour of the seed” due to absence of tannins; “ orange colour of cotyledons”; “high earliness” and “standing ability” of the plant; all in lines with different seed size.

In blue lupine the programme included the selection of most productive and adapted wild types collected in the Lazio region (2 lines out of 34 different accessions analyzed, donors of high central Italy adaptability). These 2 lines were crossed with domesticated cv developed in Australia and Poland and in the following generations were recovered all the most important domestication characters (“white flower”, “white seed coat”, “pod indehiscence”, “non hard seed”, “large seed size”, both “indeterminate” and “determinate” plant habit, “absence of bitter antinutritional factors” in the plant and in the seed, “cold tolerance” for fall seeding, high fertility and seed production.

In all 3 species several selected lines are now under multiplication and analyses of production potential are now conducted , before their release.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.31

WHOLE-GENOME SCAN FOR THE SIGNATURE OF SELECTION IN *PHASEOLUS VULGARIS* L.

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domestication, molecular markers, common bean, selection signatures

The molecular screening of the whole genome for the signature of selection is a very promising approach to identify genes and genomic regions with adaptive role. “Natural selection mapping” or “Genomic scans for the signature of selection” can be useful in order to validate the role of previously identified genes of agronomic importance or to identify genes or genomic regions without any prior information. Here, we present the map-based analysis of genetic diversity in wild and domesticated accessions of Andean and Mesoamerican *Phaseolus vulgaris* L. (common bean) using different classes of molecular markers: chloroplast SSR, AFLPs, and STS. AFLP and STS have been mapped in the *P. vulgaris* core map BAT93 x Jalo EEP558, in which several genes and QTLs of agronomic relevance have already positioned, including loci involved in the domestication syndrome. Several statistical methods were used in order to identify loci that show a pattern of diversity and divergence departing from neutral expectations. The map location of these outlier loci were compared with the positions of the genes and QTLs already available in the consensus map allowed an indirect validation of the result. Our results show that, in common bean, the genome-scan approach is a very promising approach to map and identify genes of interest.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.32

VARIATION FOR LECTIN SEED PROTEINS IN A COMMON BEAN (*PHASEOLUS VULGARIS* L.) GERMPLASM COLLECTION

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lectins, phaseoline, seed proteins, common bean., genetic diversity

In *Phaseolus vulgaris* phaseoline are the most abundant seeds storage protein and variation for their electrophoretic patterns has been used to understand the origin and evolution of this widely cultivated crop plant. Common bean seeds also contain a family of closely related proteins, the lectines, that play an important role in plant protection against predator. Plant lectines are a large family of homologous carbohydrate binding proteins that are present in different organs and tissues of many plant species and particularly in the seeds as in several legume species. In beans, lectins have molecular masses above 31 kD and genetic evidence shows that genes for lectin-related protein family, composed of arcelin, α-amylase inhibitor and phytohemagglutenin are located at the same locus.

In this study a collection of 535 European accessions of *Phaseolus vulgaris* L. from 25 countries (20 from Albania, 19 from Austria, 18 from Bulgaria, 19 from Croatia, 37 from ex Czechoslovakia, 19 from France, 28 from Georgia, 20 from Germany, 12 from ex German Democratic Republic, 17 from Greece, 8 from Hungary, 44 from Italy, 35 from Netherlands, 16 from Poland, 21 from Portugal, 24 from Romania, 23 from Slovakia, 18 from Slovenia, 27 from Sweden, 95 from Spain, 3 from ex Union of Sovietic Rocialist Republic, 1 from United Kindom, 7 from Turkey, 2 from Ukraine, and 2 from Yugoslavia) previously tested for phaseoline variation (75.5% of accessions from Andean gene pool, 'C' and 'T' phaseoline, and 24.5 % accessions of Mesoamerican gene pool, 'S' phaseoline), was analysed for SDS-PAGE lectin patterns and association among phaseoline, lectin patterns and country of origin was investigated.

Results obtained showed overall nine lectin variants ranged from 29 kD to 36 kD.

Three variants include 86.17% of accessions (48.60%, 22.43% and 15.14% respectively). Phaseolins and lectins displayed levels of association. One lectin variant did show association with 'S' phaseolin type, while a second one did show association with Andean phaseolin type and Eastern Europe countries.

Variation for lectin and phaseoline seeds protein could be useful to investigate the distribution Andean and Mesoamerican germplasm in European countries and to identify the most diverse genotypes from different genes pools to be used in breeding programs and in the study of evolutionary patterns of beans in Europe.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.33

THE USE OF AFLP MARKERS AND CHARACTERISATION DATA TO SAMPLE DIVERSITY IN A CORE COLLECTION OF PHASEOLUS VULGARIS L.

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core collection, Phaseolus vulgaris, AFLP, Phaseoline, seed trait

A core collection is a subset of accessions from a larger collection of a particular crop species that represents, with a minimum amount of repetitiveness, the genetic diversity of that crop species and its wild relatives. With limited resources, it is difficult to manage large germplasm collections. Criteria that have included geographical, morphological, biochemical and molecular data, have been used to analyze genetic diversity in order to construct core collections. DNA marker systems, which provide large number of polymorphic loci dispersed in the genome could be used efficiently for development of core collections. From base collection of common bean (*Phaseolus vulgaris* L.) including 560 accessions, a collection of 305 accessions, was sampled from different gene pools: 79.7% from European gene pool, 12.8% from South America and 7.5% from Middle America. This collection was analysed for seven seed traits (length, height, width, shape, colour, colour pattern and coat pattern- IPGRI descriptor), SDS-PAGE phaseoline patterns and E-AGT/M-GAC fluorescent AFLP primer combination. Core collections, consisting of $r = 0.18 \times n$ accessions, were sampled by several sampling strategies, simple random sampling, random-stratified by log frequency of accessions by continent, random-stratified by log frequency of phaseoline pattern, that are not dependent of molecular markers, and one marker assisted sampling that maximize genetic diversity at AFLP marker loci. An independent verification of the core collection sampling efficiency was performed using geographical, morphological, biochemical and molecular data by comparing the genetic diversity found in each core collection with the variability of base collection. The AFLP-based strategy allowed to include the largest number of AFLP loci (n=37) in the core collection, all classes for seed coat pattern and seed shape, but a smaller number of countries (n=21 out of 35 in the base collection). The no marker-based sampling strategies reduced AFLP marker loci richness from n=37 (base collection) to n=27 in the core collections.

Our results confirmed that strategies based on information obtained from marker loci lead to retention of the maximum number of loci and are useful tool for plant breeders and germplasm collection curators to better understand the genetic diversity within a germplasm collection.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.34

RECOVERY AND CHARACTERIZATION OF TYPICAL ITALIAN LENTILS

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Lens culinaris, biodiversity, local population, molecular markers, ploidy

In the last 50 years, Italian lentils suffered a dramatic reduction of both growing area and production due to constraints such as the lack of innovative agro-techniques, the high harvesting costs, the use of improved foreign cultivars, and a reduced consumption. Because of these factors, Italian local populations of lentil risk severe genetic erosion. The opportunity of introducing again this legume into the daily diet lays on its well-known healthy traits linked with the benefits of a better soil management and a dry land recovery. Among the local germplasm, Onano and Altamura lentils, coming from the homonymous locations in Latium (high Tuscia) and Apulia regions, respectively, were greatly appreciated due to their good organoleptic traits, easy cooking and superior taste. The lentil of Onano has old origins dating back to the XVI century and, since the beginning of 1900, received different prizes at several national and international exhibitions. Its present production of about 40 tonns is involving now a growing area of 57 hectares that, however, not always is referred to the same ecotype traditionally cultivated and appreciated in the area. Few farmers are sporadically growing the lentil of Altamura, exclusively for self-consumption.

The present work is aimed at identifying and characterising the true-to-type local populations of both these lentils for a safeguard and the valorisation of an Italian genetic heritage that, in the past, represented a great economical support to Onano and Altamura communities.

The lentil of Onano, grown in comparison with the Canadian cv. Eston (100 seed weight ~3 g) in different locations such as Onano, Viterbo and Rome during 2002-2004, was characterised for morpho-physiological traits in the different phases of its biological cycle. Plant height overcame of 12% the Canadian control but with a lodging of 20%. The plants, generally with semi-erect habitus, were better adapted to their typical growing environment; if winter sown, they showed a biological cycle of about 6 months that were reduced to 3-4 months in case of spring sowing. The grain had a light green colour with a 100 seed weight of ~ 5-6 g. Compared with the cv. Eston in the countryside around Altamura, the homonymous lentil revealed a vegetative habitus not much different from the Canadian variety; it showed a plant height of about 48 cm and a quite good number of both branches and pods per plant. The grain yield of both Italian ecotypes was a little lower than the cv. Eston. The large and flat grain of Altamura lentil was characterised by a 100 seed weight of ~ 7-8 g and a light green colour.

The lentil populations of Onano and Altamura together with other Italian ecotypes such as Castelluccio, Ustica and Pantelleria were submitted to a molecular characterisation by intermicrosatellites (I-SSRs). Their relationship to the Canadian variety Eston was evaluated. The ploidy level of some populations was also analysed by flow cytometry.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.35

BIOTECHNOLOGICAL STRATEGIES TO ALFALFA IMPROVEMENT

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aintegumenta, alfa-alfa, SAG12-IPT, seed size, senescence

We tried to face some aspects of alfalfa improvement: the increase of seed size to allow precision sowing, the delay of leaf senescence to reduce yield losses.

STRATEGIES TO IMPROVE SEED SIZE:
The ectopic expression of the transcription factor Aintegumenta (ANT), produces hyperplasia of organ and seeds in tobacco transgenic plants because ANT affects the duration of cell proliferation during organogenesis, but does not affect organ morphology (Mitsukami and Fisher, 2000).
In order to obtain alfalfa seeds of suitable size for precision sowing we expressed the ANT gene under the control of two seed specific promoters : USP and LeB4, that are expressed in different periods of seed development.
Plants of highly regenerable genotypes of alfalfa and *M. truncatula* were transformed with the ANT harbouring constructs and the transgenic plants obtained were analysed by Southern.
Expression and phenotypic analysis are currently in progress.

STRATEGIES TO CONTROL SENESCENCE:
Manipulation of senescence programme in alfalfa was performed expressing the IPT gene, that encodes an enzyme catalysing the rate limiting step in cytokinin biosynthesis, under a senescence specific promoter (SAG12). This strategy was shown to delay leaf senescence in model plants (Gan and Amasino, 1995).
The SAG12-IPT construct was used to transform the genotype Regen sy 27 of alfalfa via *Agrobacterium tumefaciens*.
Transgenic plants were verified by Southern analysis and expression of the transgene was checked by RT-PCR.
A preliminary evaluation was carried out growing leaf pieces of transgenic and control plants in vitro, leaves expressing SAG12-IPT construct displayed a stay-green phenotype.
Field evaluation is currently in progress.

Literature:
Gan S., Amasino R.M., Science, 1995, 22; 270(5244):1986-8.
Mizukami Y., Fischer R.L., Proc Natl Acad Sci, U S A, 2000, 18;97(2):942-7.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.36

MUTATIONS OF COROLLA SYMMETRY IN SUNFLOWER

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Helianthus annuus, floral symmetry, floral mutants, actinomorphic flowers, zygomorphic flowers

The plant family Asteraceae (Compositae) is characterized by head-like inflorescences (capitula), which are considered to be directly derived from either a contracted raceme or a racemose umbel. The inflorescence of sunflower (*Helianthus annuus*) is heterogamous, with flower heads bearing two distinct flower types. Zygomorphic flowers (ray flowers), with one plane of reflectional symmetry, are characterized by three elongated petals fused to form strap-like structures surmounting a short corolla tube. They are located in the outermost whorl of the head and are sterile, retaining only filamentous remnants of the aborted style and large flat ovaries with no ovules. Actinomorphic disk flowers (tubular flowers) are arrayed in arcs radiating from the center of the head to form distinct left and right turning spiral rows. They are hermaphrodite flowers, carrying both male and female organs. Each disk flower is subtended by a sharp-pointed chaffy bract and it consists of an inferior ovary carrying a single ovule, two pappus scales (highly modified sepals) and a 5-lobed tubular-like corolla. The five anthers are joined together to form a tube, with separate filaments attached to the base of the corolla tube. Inside the anther tube is the style, terminating in a divided stigma with receptive surfaces in close contact in the bud stage before the flower opens. Two mutants with altered corolla symmetry have described. The *Chrysanthemoides* (*Chry*) mutant is characterized by a shift from the polysymmetric corolla of disk flowers into a monosymmetric ray-like corolla. Genetic analysis revealed the *Chry* phenotype to be controlled by a semidominant mutation. In Asteraceae, the bilateral symmetry of ray flowers to form abaxialized zygomorphic patterns (0:5 and/or 0:3) could be evolved either as a result of loss of an adaxial identity gene activity, such as *CYCLOYDEA*- and/or *DICHOTOMA*-like, or overexpression of an abaxial identity gene such as *DIVARICATA*-like. The *Chry* mutant, result in a capitulum comprising only ray-like flowers. This might be explained in terms of an extension of *DIVARICATA*-like gene activity into the central dome of the head. The *tubular ray flower* (*turf*) mutant is characterized by a shift from the zygomorphic corolla of ray flowers into a nearly actinomorphic tubular-like corolla. Genetic analysis of *turf*, showed that a single nuclear recessive gene controls the trait. Furthermore, we characterized in detail the morphological floral features of *Chry* and *turf*, demonstrating that both mutations also affect the development of stamens and carpels. Most disk flowers found in the peripheral whorls of *Chry* heads, showed drastic reduction in stamen length, as well as absence of ovules, and developed an unbranched style. By contrast, tubular-like ray flowers of *turf* achieved the ability to differentiate both fertile stamens and ovules. Homeotic transformations were also identified in the tubular-like ray flowers of *turf*, affecting both filaments and anthers that displayed petaloid-like traits. It is tempting to speculate that in sunflower, a regulatory network should exist between genes with a key role in the programming of corolla symmetry (i.e., *TURF* and *CHRY*) and floral organ identity genes. This interaction could be related to expression domains of *TURF* and *CHRY* not restricted to the corolla region, but extended to stamen and/or carpel primordia.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.37

INTRA- AND INTER-SPECIFIC VARIABILITY IN THE GENUS HELIANTHUS AS ASSESSED BY IRAP MARKERS

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Helianthus, intra-specific variability, IRAP, LTR-retrotransposon, sunflower

Sunflower breeding began in Russia in the 19th century using relatively few American genotypes introduced into Europe by early Spanish explorers and in Russia by Peter the Great in the 18th century. This small number of genotypes was the starting material for subsequent breeding, as has been shown by pedigree analysis of modern cultivars, even in North America. The narrow genetic background of cultivated sunflower has been a concern for its potential for improvement, and efforts to widen its genetic base are underway. Wild *Helianthus* species often constitute the basic genetic stock from which cultivated sunflower originated, and they are used for variability rescue and the introgression of important traits in *H. annuus*. The 49 North American wild *Helianthus* species have long survived extreme environments and possess resistance or tolerance genes to salt, drought, insects, diseases, as well as cytoplasmic male sterility. Gene transfer from wild species into the cultivated background largely depends on the success of interspecific hybridisation, F1 fertility, chromosome pairing for genetic recombination, efficient screening methods and a sufficient number of progenies for selection. When used in crosses with *H. annuus*, wild annual *Helianthus* species generally produce F1 seeds, perennial species do not. Such difficulties are obviously absent in crosses involving wild *H. annuus*. If wild populations of *H. annuus* indeed show large genetic variability, they could be the best genetic resources for sunflower improvement, together with or alternative to interspecific crosses.

Microsatellite and dehydrin-encoding sequence analyses suggest a remarkable genotype variability among wild sunflowers. In our experiments we have analysed genetic variability as related to retrotransposon sequences within *H. annuus* using the IRAP technique. According to IRAP (Inter-Retrotransposon-Amplified-Polymorphism, Kalendar et al. 1999) protocol, one or two primers designed on retrotransposon LTRs are used for PCR amplification of fragments of adjacent retrotransposons.

We have analysed IRA polymorphism in 30 accessions of wild sunflowers (*H. annuus*), 7 sunflower cultivars randomly chosen according to their different provenance (one for each country in which sunflower is a major crop, hence presumably not deriving from the same inbred lines), 18 *Helianthus* species, and *Viguiera multiflora e Tithonia rotundifolia.* Larger variability was observed, as expected, among wild *H. annuus* accessions compared to sunflower cultivars. Interestingly, the extent of variability of wild sunflowers was similar or even larger to that among *Helianthus* species, i.e. intraspecific was larger than interspecific variability. Phylogenetic analysis showed that *H. annuus* accessions do not belong to a single clade. On the contrary, they are in loose order among *Helianthus* species in the tree.

The large extent of retrotransposon variability within *Helianthus* observed in our experiments indicates lack of coevolution between retrotransposons related to selected primers and the host species. Such a lack of coevolution is probably due to massive activity of retrotransposons after *Helianthus* speciation. It is known that different environmental conditions can activate retrotransposons. The massive activity of

retrotransposons could be related to the different geographic distribution of wild *H. annuus* and *Helianthus* species in North America.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.38

MOLECULAR MARKER-ASSISTED CHARACTERIZATION OF MULBERRY (*MORUS* SSP.) CULTIVARS FOR THE CONSTITUTION OF A CORE COLLECTION

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AFLP markers, core collection, mulberry germplasm, genetic diversity statistics, cluster analysis

Mulberries are members of the genus *Morus* L., a taxonomic group showing a great genetic variability and adaptability to different environmental conditions. This study deals with the use of AFLP-based fingerprints as a tool for estimating genetic variability within as well as among three different mulberry species (i.e., *M. alba* L., *M. latifolia* Poir. and *M. bombycis* Koidz.) Preliminary flow cytometric analyses pointed out the presence of diploid as well as triploid accessions. A high level of polymorphism (72.2%) was found over all the 48 accessions analyzed. Genetic similarity (GS) within single *Morus* species ranged from 0.845 (*M. bombycis*) to 0.884 (*M. alba*) being intermediate in *M. latifolia* (0.869). The between-species mean genetic similarity estimates based on pair-wise AFLP marker fingerprint comparison were very similar ranging from 0.861 to 0.874. The partition of the genetic variation over the three *Morus* species was unexpected: a proportion of the among-species genetic diversity as low as G_{ST}=0.084 pointed out that about 92% of the total genetic diversity found among *Morus* accessions is due to DNA polymorphisms within a species, while only 8% of the total variation was highlighted among species. Our data indicate that some of the introduced accessions showing distinctive phenotypes, clearly differentiated from those revealed in the original habitat where they have been selected and adapted, hide an identical genotype. Current studies are aimed to set up a high reproducible identification method on the basis of accession-specific AFLP marker sequences to be used in simple PCR-based haplotyping.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.39

TY1-*COPIA* RETROTRANSPOSON-BASED S-SAP MARKER DEVELOPMENT IN *CYNARA CARDUNCULUS* L.

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retrotransposons, S-SAP, globe artichoke, wild and cultivated cardoon

Long terminal repeat (LTR) retrotransposons are a class of mobile genetic elements that have been harnessed for the development of molecular markers in plants. One of the most popular and applied system to exploit the distribution of the LTR retrotransposon is the S-SAP technique. The only disadvantage of the S-SAP markers is the need to acquire a retrotransposon LTR sequence from a given species. As LTRs do not contain any conserved motifs, to isolate the sequence information it is necessary to perform an amplification between a conserved region in the RNAseH gene and a restriction site in the adjacent LTR sequence or flanking genomic DNA

Recently, we developed a transposons display assay based on a low copy number LTR-retrotransposon (CYRE-39) in *Cynara cardunculus* L; the species includes wild cardoon [var. *sylvestris* (Lamk) Fiori, cultivated cardoon [var. *altilis* (DC)] and globe artichoke (var. *scolymus* L.). The latter represents an important component of the European agricultural economy, crop production being in excess of one million tons and 100,000 ha in cultivation.

Here we report on the isolation and development of S-SAP assay for the analysis of distribution of high copy retro-elements in *C. cardunculus* . We applied a modified protocol derived from the one previously developed by Pearce and co-workers (1999; Plant Journal 19: 711-717). We successfully isolated three sequences containing LTR and RNAseH specific domains. They were named CYRE-5, CYRE-10 and CYRE-13; two primers CYRE-5for and CYRE-13for were derived and used for S-SAP analysis performed on 20 artichoke accessions, one cultivated and one wild cardoon. Six primer combination were applied and 124 polymorphic bands obtained with an average number of 20.6.

S-SAP data were compared with the ones obtained by applying 9 AFLP primer pairs, which originated 256 polymorphic bands (28.4 on average). Cluster analysis derived from the two marker systems showed congruent results. The S-SAP assay developed on CYRE-5 and CYRE-13 proved to be effective for the analysis of retrotransposon-based DNA polymorphisms in *C. cardunculus;* our ongoing research is addressed at positioning CYRE markers in a molecular genetic map of the species which is under construction.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.40

CHARACTERIZATION OF A RED CHICORY INTERCROSSING POPULATION FOR MORPHOLOGICAL AND MOLECULAR TRAITS

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red chicory, descriptors, phenotipic traits, AFLP

Red or variegated chicory represent valuable high-quality Italian crop that is acquiring more and more commercial interest. This vegetable, locally called "radicchio", includes different types, which differ both for head shape and leaf colour. In this species is difficult carry out genetic studies for the allogamous mating system and the presence of sporophytic incompatibility. Selfing is also limited by unfavourable flower morphology and a strong gametophytic competition between self and crosspollination. At present commercial varieties are obtained from synthetic populations. Breeding programmes are developed to select the best local lines to combine in new varieties. The aim of this work was to analize an intercrossing population for traits related to morphological uniformity and to perform an AFLP molecular analysis to characterize these lines. Specific descriptors were generated to define characters difficult to measure precisely. In fact, most of traits of interest, including leaf shape, leaf colour, head shape, heading capacity and rib thickness, showed a continuous variation, strongly influenced by environmental conditions. A subset of individuals that represent the most extreme phenotypes in the population were identified and molecular analysis to find association between phenotypic traits and molecular markers has began.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.41

ASSESSING MOLECULAR DIVERSITY ON GRAPE (*VITIS VINIFERA* L.) GERMPLASM FROM BASILICATA REGION

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Aglianico del Vulture, ancient grape germplasm, molecular markers, genetic polymorphism

Wine production is a key agricultural activity in Italy, covering a significant area of arable land. Producers of variety wines have been able to improve the technical quality of their wines, due to the natural environment and /or to the more efficient farm structure. It is ascertained that the characteristics of each wine depend by different factors, such as the pedoclimatic condition, the grape must composition and the technological applications. Among all these factors, the wine typicality can be strongly influenced by the grapevine cultivar and the yeasts which have performed the fermentation process (Fleet, 1990 – J Wine Res *1*: 211-223).

The increasing interest to evaluate the natural genetic variability of each ecosystem has stimulated the development of approaches of molecular methods with the aim to advance significantly this knowledge. Nowadays different molecular methods are available to identify and characterize both grapevines and yeasts.

On the other hands, an high risk of genetic erosion in grape germplasm is determined by the spreading utilization of national and international grape cultivars in many areas of Mediterranean region typical for this species, included the south Italy and in particular the Basilicata region.

The grapevine (*Vitis vinifera* L.) is a clonally propagated crop and several morphological markers have been used for the characterization of plant germplasm, clarifying ambiguous denominations, but these methods are based on characters which can be highly affected by the environment.

The methods based on genetic variation have frequently been used for these purposes with more or less success, depending on the genetic relationships among the materials analysed and the number of markers employed. Isozyme and molecular markers, such as RAPD, RFLP, microsatellite and AFLP have been used on *Vitis vinifera* in several studies in order to discriminate among grape cultivars. However, few researches based on molecular markers and focused on the genetic variation within different clones of the same cultivars showed sufficient resolution to identify with a specific cultivar or to distinguish clones from somatic mutation or clonal selection.

The present research show a first survey on three typical grapevine areas of Basilicata region in order to retrieve and collect ancient grape clones mainly produced in these specific geographical areas : Vulture, Valdagri and the hilling area of Matera.

In the present paper starting from about 150 presumed clones, the genetic variability among genotypes were assessed based on different classes of molecular markers (microsatellites, AFLP).

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Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.42

INTEGRATED SNPS-BASED GENETIC MAP OF GRAPEVINE (*VITIS VINIFERA* L.)

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functional map, SNPs, grapevine, anthocyanin metabolism, berry colour

Fine mapping is the basic work to develop markers suitable for marker assisted selection programs and map based cloning approaches. To this purpose, several international groups have developed a consistent number of molecular maps in the last few years, mainly based on co-dominant (microsatellites) and dominant (AFLP, RAPDs) markers. SSRs are the essential basis for cross-talk between maps and represent the most useful markers due to multiallelism and reproducibility. A basic number of SSRs used for several referring grapevine maps have been used to build up a framework of co-dominant markers conferring to any growing map an universal value. Dominant markers are useful only when referred to a specific cross but it is difficult to export them to other crossing population analysis. The main objective of the present work was to identify rapid and economical methods to score for co-dominant single nucleotide polymorphimsms (SNPs), possibly applicable to automatic or semi-automatic procedure. As a second goal, the attention has been focussed on SNPs development based on internal or international EST databanks and scored on the crossing population Freiburg 993-60 (complex hybrid between *V. vinifera, V. rupestris* and *V. lincecumii*) x *Vitis vinifera* cv. Teroldego. In particular we focalised our attention to the genes belonging to anthocyanin metabolism. We mapped these genes and the transcription factor involved in their expression. The colour of the berry segregates with this gene demonstrating the correlation between MybA and anthocyanin biosynthesis.

Due to the large synteny within Vitis spp., positioning of EST-based SNPs confer to the described data an universal value in grapevine mapping. So this first map based on SSRs and SNPs represents a stimulus to any future mapping work demonstrating the synergy between multiallelic anonymous markers as SSR and biallelic gene-specific markers based on SNPs.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.43

CHARACTERISATION OF LOCAL NORTH-EASTEARN ITALIAN GRAPEVINE CULTIVARS USING MICROSATELLITE AND FUNCTIONAL MARKERS

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grapevine, SSRs, local varieties

Grapevine (*Vitis* spp.) includes cultivars, wild species and hybrids that show a wide genetic variability. Grapevine cultivation is an ancient tradition in the Veneto region, and therefore a large number of ancient cultivars are still present. Some of these (e.g. Raboso and Prosecco) are recognised as producing important Controlled Designations of Origin (D.O.C.) wines, while other local types have been lost because substituted by international cultivars.

Our aim is to characterise these ancient grapevine cultivars grown for centuries in the North-Eastern Italy to preserve their genetic variability. To this purpose we are characterising 30 cultivars with microsatellite markers, some of which are recommended by the GENRES No. 81 EU Project for the identification and differentiation of the European grapevine cultivars or varieties. Due to different labelling used by various laboratories, direct comparison of microsatellite size data is often difficult. Therefore the transformation procedure proposed by GENRES No. 81 is being used to add our local cultivars to the European database. Further marker descriptors, chosen on the basis of our preliminary examinations, are also used. The results indicate the possibility to differentiate the varieties on the basis of their individual SSR pattern and allele sizes. The phylogenetic relationships among the cultivars are also evaluated. A functional characterisation is in progress by evaluating the sequence and expression variation of grapevine MybA genes in relation to berry colour.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.44

GENETIC VARIATION AT MARKER LOCI IN AN ITALIAN COLLECTION OF WILD AND CULTIVATED GRAPEVINES (*V. VINIFERA* L.)

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genetic resources, SSR markers, Sex locus, Vitis vinifera spp silvestris

Analysis of variants that are found in nature provides an important source of genetic variation that can be used to gain insight into the control of important processes in plants. In many cases, including *Vitis vinifera*, natural variation present among accessions is multigenic, which has historically hampered its analysis.

Phenotypic variation for morphological and physiological traits is abundant in cultivated grapevine and enables almost every *V. vinifera* variety to be distinguished from other varieties. Unfortunately, wild grapevine individuals (*V.v.* ssp. *silvestris*) have been scarcely explored and natural populations are today reduced to a very low size.

In order to improve both management and exploitation of our germplasm collection, we started genotyping every accession of cultivated and wild grapevines at the six most polymorphic and widely used SSR loci. Moreover, suitable map based SSR markers have been included to analyse the allelic variation at certain phenology quantitative trait loci while characterizing the QTLs with candidate genes and experimental segregating populations.

We then investigated the genetic diversity, structure and differentiation within 160 Italian grape cultivars and 210 accessions of wild grapevines collected throughout Italy in the past years.

Since ssp. *silvestris* can principally be distinguished from cultivated grapevine by its dioecism, these accessions were further investigated at the sex-determining locus recently mapped by our group in *Vitis riparia* linkage group 2. The genetic relationship between wild and cultivated grapes and the pattern of variation at four SSR loci linked to the sex gene will be presented.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.45

CHARACTERISATION OF OLIVE GERMPLASM FROM ABRUZZO REGION BY MICROSATELLITE MARKERS

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Olea europaea, microsatellites, germplasm, SSR, DNA

In the whole Mediterranean basin a large number of varieties of *Olea europaea* L. are present. This produce a series of problems concerning the germplasm characterization, management and preservation. In addition there is the problem arising from the existence of homonyms and synonyms. This make difficult the cultivar identification. The single sequence repeats (SSR) are co-dominant markers, showing a large number of polymorphisms *per primer* set and often multiple alleles in a variety, which can be highly informative.

In this work 23 accessions of olive from the Italian germplasm collection of the *CRA Istituto Sperimentale per l'Olivicoltura at Rende, Cosenza, Italy* (CRA-ISOl), were studied, corresponding to the major part of the autochthon germplasm of Abruzzo region: Caprina di Casalanguida, Caprina Vastese, Carbonchia, Carpinetana, Castiglionese, Crognalegna, Cucco, Dritta, Gentile dell'Aquila, Gentile di Chieti, Intosso, Nebbio di Chieti, Nebbio di Pescara, Olivastro di Bucchianico, Olivastro Frentano, Pescarese, Posola, Posolella, Precoce, Puntella, Rustica, Toccolana and Tortiglione.

Thanks to the identification and characterization of 23 olive cultivars with unique genotype we can affirm that SSR technology is an efficient tool for genotyping the olive germplasm collection of Abruzzo region and could be valid to distinguish other accessions which can be introduced into the collection.

This study showed that the use of molecular markers like SSRs is very useful to build a data base available for variety analysis and for olive germplasm collection management.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.46

IDENTIFICATION OF TWO PUTATIVE CHS ISOFORMS IN *OLEA EUROPEA*

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Olea europea, Chs, molecular markers

Flavonoids are important secondary metabolites directly involved in the interaction between the organism and its environment (Winkel-Shirley, 2001). Plants synthesize a variety of flavonoid compounds that are the major flower pigments and also play important roles in defending plant against pathogens, in acting as signal molecules in plant-microbe interaction and in protecting plants from UV radiation (Harborne, 1994; Shirley, 1996).

The chalcone synthase gene (Chs) codes for the branch point enzyme of the flavonoid pathway, hence it may play a role in adaptative evolution. Chs is encoded by a single copy gene in some plant, such as *Arabidopsis* (Koch et al., 2001) and by multiple isoforms of the gene in others, such as members of *Asteraceae* (Yang et al., 2002).

In *Olea europea*, only a part of the Chs coding sequence has been sequenced and deposited in GenBank DNA database at the moment. The present study was conducted to isolate sequences of the Chs gene, with the aim to develop molecular markers from polymorphism for cultivars characterization.

Results obtained allowed the identification of two different sequences which are upstream to the known coding sequence and 2002 and 1466 bases pair long respectively. These sequences showed high identity to hortologous genes deposited in GenBank DNA database. In particular some portions of them showed a high identity values (80-83%) to exon I and II of the Chs isoforms gene of *Vitis vinifera*. On the contrary, low identity values to 5' UTR region and to the intron have been found. However these results seem to suggest that the isolated sequences in *Olea europea* include a portion of 5' UTR region, the exon I, an intron and a newly sequenced part of the exon II of the Chs gene. Moreover the alignments between the portions corresponding to the exons of the two isolated sequences showed high identity values (91-92%), on the other hand the regions corresponding to the introns showed low homology (10%). These results suggest that the two identified sequences could be two different isoforms of the Chs gene in *Olea europea*.

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Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.47

DEVELOPING SNP MARKERS FROM CANDIDATE GENES IN OLIVE

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Single Nucleotide Polymorphism, Olea europaea, PAL, ACP, actin

The identification of SNP (Single Nucleotide Polymorphism) markers has assumed a particular relevance in olive genetics for the screening of the germplasm resources, for mapping purposes and for varietal discrimination as well.

The identification of SNPs has been oriented toward specific coding sequences involved in the phenylpropanoid and fatty acid synthesis and in other important pathways.

For the identification of the olive candidate genes degenerated primers, designed on conserved regions of the genes previously identified in other species, were used for PCR amplification of the homologous in olive cDNA, cv. Leccino. After cloning, the Genome Walking technique was adopted and sequences were extended on both sides of the clones, in order to recover the full-length gene. To identify potential SNPs several portions of the genes were directly sequenced on a restricted number of cultivars. Once a polymorphic region was discovered, a systematic direct sequencing of that region on a wider set of cultivars was performed.

On a region of the phenylalanine ammonia-lyase (PAL) gene (714 bp), 3 SNPs and 1 indel were screened.

From the analysis of a 932 bp fragment of the Acyl Carrier Protein (ACP) gene containing a 684 bp intron 5 SNP and 1 indel of 10 bp were identified.

An actin gene has also been identified and the presence of 5 SNPs was retrieved from a 426 bp fragment.

Most of the SNP identified were heterozygous and, as expected, the intron regions have shown the highest variability. For the 3 regions analysed a SNP every 138 bp has been encountered.

The identification of the corresponding aplotypes has been undertaken on a set of 10 cultivars.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.48

HIGH-RESOLUTION DNA MELTING CURVE ANALYSIS TO ESTABLISH *PHYA* GENOTYPIC IDENTITY WITH SATURATING DNA DYE IN *OLEA EUROPAEA* L.

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Olea europaea L., HR-melting analysis, LCGreen ™I, phytochrome A, SNP

The importance of phytochrome A (PHYA) in plant development has long been recognized. Current studies continue to show a correlation between degree of mutation of *phyA* gene and the regulative role into signal transduction of PHYA. *phyA* has been used in the evolution study of Angiosperms and recently as molecular functional marker for genotyping assay of many cultivated and spontaneous species. Therefore, it is a good candidate as molecular marker in biodiversity analysis, germplasm collections typing and products traceability. Genotyping *O. europaea* is particularly complex due to the broad number of cultivars and the analysis is complicated by the presence of synonymy and ambiguous cultivar assignments. The new challenge in plant genotyping is SNPs assay, which is robust and specific, although the identification of single mutations and the analysis are laborious and expensive.

In this study, sequence portion of *O. europaea phyA* gene has been obtained by PCR amplifications using degenerate primers, designed on an alignment of all the genes present in GenBank. The obtained PCR product of cv. Canino was cloned and sequenced (Accession: AY924378). All subsequent PCR amplifications were performed on 14 *O. europaea* cultivars from different latitude (southern to central region of Italy) and habit, using specific primers that amplified a 307 bp fragment. The DNA templates were used at 50 ng under saturating LCGreen™I fluorescent DNA dye conditions and the reactions were performed with LightCycler (Roche). Melting analysis was performed either on the LightCycler immediately after cycling and on high-resolution melting instrument (HR-1), subsequently. Using LigtCycler and HR-1 softwares we performed continuous acquisition of fluorescence until 89 °C. Normalized and derivate melting curve were calculated by software.

Shape of melting curve and value of Tm were able to distinguish and separate every amplicon of each cultivar. Using the values of Tm we were able to grouped in three main cluster all the genotype. The first cluster identified the most part of cvs from Sicily and Calabria, plus Bardhe di Tirana, and Nociara, which is from the southern part of Apulia (Taranto, Brindisi). The second cluster grouped together the cultivars belonging to the Central region of Italy and those from the region located at the North of Pollino mountains. The third cluster included only *O. europaea var. sylvestris.* Variation in melting curve shape were also present in some of the cultivars indicating that an allelic condition of the gene is present. To validate the obtained results with this technique the *phyA* portion has been sequenced in all cultivars, in order to uncertain if the same haplotype belongs to the same group have high value of homology.

In this work we show the possibility to use this technology to identify probably mutations in unknown sequences as first step of SNPs recognition, through the high melting curve shape and Tm value. Moreover, this technology is also able to identify heterozygotes by a change in melting curve shape.

We present for the time results related with the application of this technology to plant genomic analysis. Finally, now it is available a robust and specific assay, easy to apply and reliable for identification of polymorphisms among genes and cultivars both as first screening of unknown sequences and for SNPs analyses of large number of genotypes.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.49

MOLECULAR CHARACTERIZATION OF *OLEA EUROPAEA* L. CV. "ARBEQUINA" CULTIVATED IN CORDOBA PROVINCE (ARGENTINA) USING AFLP MARKERS

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Olea europaea, intra-varietal diversity, AFLP markers

The area of olive growing in Argentina is limited to Western and Centre regions of the country. In the later years, there has been a general trend of increased olive plantation in Argentina, and, at present, more than 40.000 ha of new olive trees are projected to be planted, most of which are being established away from the traditional olive production area.

Olive production of the Córdoba province is localized principally in the Cruz del Eje Department and the Traslasierra Valley. The predominant variety is "Arbequina", representing 70 % of the Córdoba patrimony. This variety has been intensively propagated in Córdoba province in the past since it is well adapted to climate conditions.

Argentinean cultivars have been maintained and propagated mainly vegetatively by cuttings using the plant material locally available. To face the present olive oil demands Argentinean olive orchards need to be renewed and restructured. The renewal of Argentinean olive orchards should be performed using certified olive trees of the selected Argentinean olive cultivars.Therefore, the analysis of genetic diversity is essential for the correct acquisition, maintenance and use of genetic resources.

The main objective of this study was to investigate and analyse genetic intra-cultivar diversity of 38 accessions of variety "Arbequina" by AFLP markers. The accessions were collected from Traslasierra Valley and Cruz del Eje location, Córdoba province, Argentina. Moreover, three "Arbequina" cultivars ("Arbequina - Argentina", "Arbequina – International" and "Arbequina – Spain") were included in the screeningand use as control. This material was provided by the collection of the Olive Research Institute, CNR, Perugia (Italy).

Only clearly polymorphic and reproducible bands were scored as markers: present = 1 or absent = 0. Genetic similarity was calculated using the Dice' index (1945). A dendrogram was constructed from the Dice' similarity data with NTSYS-PC software by unweighted pair-group means cluster analysis (UPGMA).

A reasonable number of scoreable bands per gel were obtained with the EcoRI/MseI primer combinations. The 19 primer combinations used to perform the AFLP analysis have produced a total 95 unambiguous bands.

The dendrogram shows a rather high variability within the cultivar examined and it is composed by six main clusters. The range of intravarietal similarity is from 0.88 to 0.95 among accessions studied.

The majority of accessions is included in the same cluster independently to the growing area. The “Arbequina - Argentina” and “Arbequina – International” cultivars were included in this last group while “Arbequina – Spain” cultivar was grouped in an other cluster together with the remainder accessions. Probably, this pattern suggests that the accessions of variety “Arbequina” may have developed genetically distinct clones over fifty years of cultivation. These clones would not necessarily show phenotypic differences and the molecular variability could be present within of them.

However, the results of the present study do suggest that there is some diversity among accessions, which can be used for selection of potentially superior clones for future olive improvement in the Córdoba province.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.50

MEIOTIC ABNORMALITIES IN PMCS OF DIFFERENT *CITRUS* POPULATIONS

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cytomixis, fertility, meiosis, chromosome association, Citrus

A cytogenetic study undertaken to determine the mechanisms responsible for reduced fertility in some *Citrus* selections revealed the presence of several meiotic abnormalities in microsporogenesis of genotypes characterized by a different genetic basis. In particular, cytomixis (i.e., chromatin migration between adjacent meiocytes through cytoplasmic channels) was found to occur in pollen mother cells (PMCs) of diploid selections and hybrids and in two tetraploid genotypes obtained by symmetrically interspecific somatic hybridization. Single and double chromatin bridges between two or more adjacent meiocytes were mainly observed at prophase I, but also in the subsequent phases of first division and in the second meiotic division, in the latter case at a low frequency. The percentage of cytomictic microsporocytes varied from 3% in metaphase I to 17.7% in early prophase I in the diploid populations, and to 20% in diakinesis in one of the two tetraploid somatic hybrids. Moreover, in both somatic hybrids several microsporocytes were totally empty as a result of complete chromatin migration either into another meiocyte or occasionally outside the cell. Such meiocytes are very likely to get lost during meiotic division. No evidence of cytomixis was found in the control plant.

In addition to cytomixis, still other meiotic abnormalities were observed in *Citrus* PMCs, in both the first and second meiotic divisions. The most common of such abnormalities were those related to irregular chromosome segregation. Cells with a polyploid chromosome number were also observed as a result of endoreduplication which can lead to the formation of 2n and/or 4n gametes.

The occurrence of cytomixis and other meiotic abnormalities was analyzed in comparison with pollen viability. The plants characterized by a great number of cytomictic microsporocytes showed lower values of pollen viability compared to the plants with little or no trace of cytomixis and to the control. A possible relationship between the occurrence of cytomixis and other meiotic abnormalities, and reduced fertility in the *Citrus* populations is discussed.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.51

MOLECULAR CHARACTERIZATION OF NEW CITRUS TETRAPLOID HYBRIDS BY MEANS OF SSR MARKERS

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protoplast fusion, seedless, lemon, mandarin, flow cytometry

One of the most important goal in Citrus genetic improvement is the obtainment of seedless cultivars. Among the available strategies to generate seedless cultivars, the most used one takes into account the accomplishment of suitable interploid crosses (2X x 4X). In Palermo, at the Research Division of the Institute of Plant Genetics, advanced breeding techniques were applied to foster the raising of seedless cultivars in lemon (1) and mandarin (2); the following crosses have been planned: (1) three allotetraploid somatic hybrids (4X), "Milam (purported hybrid of *Citrus jambhiri* Lush) + Femminello (*C. limon* L. Burm. f.)"; "Valencia (*C. sinensis* L. Osbeck) + Femminello"; "Key lime (*C. aurantifoglia* Swingle) + Valencia") have been used as pollen parents in crosses with diploid 'Femminello' lemon (2X); (2) autotetraploid (4X) 'Dancy' mandarin (*C. reticulata* Blanco) was used as pollen parent in four crosses, respectively with: 'Duncan' grapefruit (*C. paradisi* Macfadyen), 'Wilking', 'Ortanique' and 'Fortune' mandarins (*C. reticulata* Blanco). To recover zygotic embryos, the embryo-rescue technique was applied 105 days after pollination. In order to distinguish 3X zygotic embryos from nucellars (2X), ploidy discrimination on all the recovered plantlets was accomplished by flow cytometry. Surprisingly, among the analyzed plants tetraploid genotypes were found. To try to understand their genetic origin, microsatellite analysis (SSR-Simple Sequence Repeats) was carried out. 7 SSR primers showed that the parental plants were heterozygous for at least two alleles (named high and low according to their molecular size), which were expected to segregate in a mendelian fashion in the sexual progeny. Some of the tetraploid plantlets showed genetic segregation for both loci, confirming their zygotic origin. To explain the obtainment of a tetraploid progeny in interploid crosses (2Xx4X), two hypothesis can be advanced: a) unreduction of megaspore; b) anomalies in the microsporogenesis process and/or zygotic formation.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.52

STEPS TOWARDS THE PRODUCTION OF A FUNCTION MAP IN PEACH (*PRUNUS PERSICA*)

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Prunus persica, Expressed Sequence Tag (EST), Single Nucleotide Polymorphism (SNP), ESTree db, Candidate gene (CG)

Peach is currently considered a model species for genomics studies in Rosaceae. An international effort is aimed to the improvement of the available EST collections, to the sequencing of gene rich regions and to the production of high-density maps, for integration of QTLs, monogenic traits and functional maps.

We report the development and mapping of genetic markers based upon expressed sequence tags (ESTs) polymorphisms and the positioning of ESTs in a physical framework map for peach genome.

Based on ESTree DB (a collection of 18630 cDNA sequences originated from eight libraries), contigs and EST were selected as candidate genes (CGs) based on sequence similarity with genes relevant for fruit quality, already characterized in other related species like apple, apricot and strawberry.

To rapidly identify SNPs, the ESTs generated from six different peach genotypes (Suncrest, Bolero, Oro, Loring, Fantasia, Redhaven) and from almond were aligned by AutoSNP, a program that allows *in silico* SNP (isSNP) detection. A total of 1863 isSNP was identified and further analysis concentrated on a subset of 67 isSNPs, derived from ESTs representing genes putatively involved in important aspects of the secondary metabolism. A set of mapping populations was also obtained from various germplasm repositories (*Yumyeong* x *O'Henry, P. ferganensis x IF7310828, Bolero x Oro* and *Texas* [almond] x *Earlygold* [peach]. Experimental validation of the obtained isSNPs was performed by amplification and sequencing of the polymorphic fragments from parentals of each mapping population. In order to obtain preliminary data for the construction of functional maps, confirmed SNPs were genotyped by minisequencing in selected individuals of the segregating populations.

In a parallel approach a strategy based on the identification of contigs representing putative genes potentially affecting fruit quality was adopted using online resourced offered by the ESTree web site. SNPs were thus scanned by sequencing of amplified products from parental lines of mapping populations. SNPs scoring was conducted on segregating populations by minisequencig.

As a complementary strategy, approximately 200 ESTs were selected to be mapped on a physical framework map, by hybridization on two arrayed BAC libraries whose clones have been fingerprinted. Currently, 17 out of 46 EST which hybridized to the filters containing the BACs clones were localized on physically mapped contigs.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.53

ESTree db: AN ENGINE FOR PEACH EST RELATED INFORMATION RETRIEVAL

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ESTree db, functional genomics, peach, bioinformatics, SNP discovery

The ESTree db (http://www.itb.cnr.it/estree/) is a collection of *Prunus persica* expressed sequenced tags (ESTs). At the moment, it encompasses more than 20,000 peach EST sequences, representing ten different cDNA libraries.

The ESTree db core structure is a MySQL relational database where all the results of the sequence analysis pipeline are stored. This analysis pipeline is a fully automatic procedure that starting from raw EST sequences fills up all the tables in the database. A php-based web interface allows querying the db and creates user-friendly graphical displays on-the-fly. The result is a web site where users can access data on sequence annotation (both versus the GenBank nr db and versus the Gene Ontology viridiplantae db), view the complete BLAST outputs, retrieve links to the best blast hits, to the NiceZyme (Expasy) db and to the KEGG Biochemical Pathways db. All the sequences of the db have been assembled with CAP3 and contig alignments and graphical displays are available. Putative SNP detection has been performed with the AutoSNP program and SNP data are also displayed. The db has been furnished with a text search utility and a local BLAST utility. Downloads of sequences, contig consensus sequences and AutoSNP SNP reports have been prepared in various formats. Statistics on the db status and on matching ontologies are provided. The ESTree db is continuously growing and new features are added, including data derived from peach microarray analysis.

The ESTree db is the first web resource reporting data on putative SNP sites in peach, and will be the main repository of data obtained by the ESTree Interuniversitary Centre units, allowing the creation of a platform for easy data integration and retrieval, with the aim to provide a tool to improve knowledge on peach genomics and functional genomics.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.54

DESCRIPTION AND COMPARISON OF SOFTWARES FOR POPULATION GENETICS ANALYSES BASED ON MOLECULAR MARKERS

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software description, population genetics, molecular markers, comparison test

Molecular markers are important tools for evaluating animal genetic resources in terms of groups of individuals or populations in order to estimate genetic diversity, statistics and similarity coefficients, genetic distances, and gene flow estimates, to test for Hardy Weinberg deviations and linkage disequilibrium levels, to infer population structure looking for clustering patterns, to find out polymorphic loci for evidence of neutral or adaptive variation and to implement assignment tests useful for breed identification. Moreover, molecular markers might assist genetic variability conservation program and could be useful to define genetic traceability methods for food safety.

Aim of this study was to describe and compare some of the software programs available to elaborate molecular marker datasets for population genetics. Recently, many software programs for the analysis of molecular polymorphisms have been developed for personal computers and their powerful statistical performances and user-friendliness make an attractive alternative to the performing of spreadsheets or simpler home-made programs. The programs described in this study are softwares widely used in population genetics as TFPGA, Arlequin, Phylip, DISPAN, Genetix, GENEPOP, PROC ALLELE of SAS, GDA, POPGENE, NTSYS and Cervus. These programs were chosen for their availability, flexibility and citations in scientific reports. Although there is a large overlap in their functionality, each of them has unique features to offer. In general, these programs grew out of an individual's or a lab's immediate research need and they were developed into user-friendly software to be shared with the larger research community. Critical aspects for these software are mainly the request of specific format input files, the availability of a clear manual and the use of many details concerning the interface with the user as warning messages, the possibility to save specific settings and to run batch files. In conclusion the major features for each program will be described and discussed.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.55

SSR MARKERS AND POPULATION GENETIC ANALYSES TO SHED LIGHT ON THE REPRODUCTIVE SYSTEM OF WHITE TRUFFLE TUBER MAGNATUM

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Tuber magnatum, SSR, reproductive mode

Tuber spp. are ectomycorrhizal ascomycetes producing hypogeous fruit-bodies known as truffles. Thanks to the distinctive taste and aroma, the truffles of some *Tuber* spp. are appreciated and marketed worldwide.

Setting aside the beneficial effects of the mutualistic symbiosis on forest tree growth and nutrient uptake, many attempts have then been made to promote the cultivation of these fungi through the use of nursery-inoculated mycorrhizal plant and fulfil their market demand. Encouraging truffle productions have been obtained for some species such as *T. melanosporum* Vittad. Conversely, the finest white truffle *Tuber magnatum* Pico is still harvested exclusively from natural truffle grounds. Estensive research work is needed not only to understand ecological requirement specific to each species/strain but also to shed light on basic aspects of truffle biology, such as their reproductive system.

The few studies of genetic structure and mode of reproduction of *T. magnatum* have reported a very limited intraspecific variation and an apparent absence of heterozygous individuals (3, 4). Based on the assumption that truffles are formed by diploid/dikaryotic hyphae these results have been explained as a consequence of a strict self reproductive system in *T.magnatum* as well as in *T. melanosporum* (1 e 2). However, the fruit bodies of most ascomycetes are formed by uniparental - haploid hyphae.

In the present study we aimed to shed light on reproductive mode of *T. magnatum* by using microsatellite markers (5) and an extensive truffle sampling (316 specimens grouped into 26 populations) to evaluate the occurrence of a genetic exchange within and among populations. Pairwise and multilocus linkage disequilibrium analyses revealed the existence of an extensive gene flow within local populations (6). We interpreted these data to mean that *T.magnatum* is an out-breeding species and its fruit bodies are mainly formed by haploid hyphae.

This study provides the first evidence suggesting that truffles are not strictly selfing organisms and should result in a significant reevaluation of the life cycle and biology of *T.magnatum* and other *Tuber* spp.

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Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.56

CYTOGENETIC COMPARISONS AMONG APIACEAE USING FLUORESCENCE *IN SITU* HYBRIDIZATION

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rDNA, FISH, cytogenetics markers, chromosome identification

Chromosome locations and distribution patterns of the ribosomal genes were assessed in several Apiaceae using double-colour fluorescence *in situ* hybridization (FISH). *Apioideae* species of economic importance (*Anethum graveolens*, *Carum carvi*, *Cumin cyminum*, *Daucus carota*, *Foeniculum vulgare*, *Pastinaca sativa*, *Petroselinum crispum*, *Pimpinella anisum*, *P. saxifraga*) and some of their wild relatives (*e.g. Orlaya grandiflora* and nine *Daucus* species) were included in the study. Interspecific variation was found both for number and chromosomal location of the rRNA gene loci. The number of chromosome pairs bearing NORs varied from one (*e.g. D. carota*, *P. crispum*) to two (*e.g. P. sativa*, *C. cyminum*) to four (*D. guttatus*). Three 45S (NOR) hybridization signals were visible in *O. grandiflora*, two of which located on the short arm of one pair of homologous subtelomeric chromosomes, and the odd weaker signal in hemizygous condition was detected on the short arm of one metacentric chromosome. One chromosome pair carrying the 5S site was detected in all the species, except in *P. saxifraga*, where four 5S sites were observed.

Linkage association between 5S and NOR sites was observed only in *Carum carvi*. The distinctive hybridization sites of the rRNA genes provided useful cytogenetic markers for the identification of several chromosomes. Based on arm ratio and chromosome landmarks, tentative karyotype description for several of the species will be presented.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.57

FINGERPRINTING OF *ANEMONE CORONARIA* CULTIVARS BY AFLP MARKERS

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breeding strategies, genetic variation, molecular markers, ornamental crops

The genus *Anemone* (*Ranuncolaceae*) includes many ornamental species, cultivated as garden plants or for cut flower production. Among them, *A. coronaria* is one of the most commonly grown. The species, in nature, is cross-pollinated by insects; self-pollination is practicable but progenies suffer inbreeding depression. Commercial seed is usually produced by crossing selected and highly heterozygous plants. Seed stocks of a sub-cv. are thus set up of a mixture of seeds obtained from a few crosses, and originate a population of plants showing phenotypic variation and poor uniformity.

Aims of this work were: (i) to define the conditions that yield distinct and repeatable AFLP profile in *A. coronaria*, which posses a wide genome (1C content = 8,45 pg); (ii) to assess the genetic variation among and within diploid and tetraploid cvs and sub-cvs.

Restriction was performed combining one eight base cutter (*Sbf*I) with a four base cutter (*Mse*I) enzymes. Six primer combinations, with 5 selective nucleotides, were chosen on the basis of clearness and reproducibility of electrophoretic patterns. A total of 152 polymorphic bands (37.0% of the total amplified bands), ranging from 80 to 700 bp, were scored. The average number of polymorphic bands per primer combination was 25.3 ranging from 19 to 32 per priming pair. The UPGMA dendrogram, generated using the Simple Matching Coefficient, grouped the *A. coronaria* genotypes in three main branches: A, which included the cv Cristina; B, which embodied two main clusters: B1 (Monalisa) and B2 (Mistral). Similarly, branch C included clusters C1 (Wicabri) and C2 (Tetraelite). The co-phenetic correlation coefficient (r-value) between the data matrix and the co-phenetic matrix for AFLP data was 0.91, suggesting a very good fit between the dendrogram and the similarity matrix from which it was derived. The hierarchical analysis of variance (AMOVA) showed a high degree of differentiation within sub-cultivars (approximately 42% of total variation,); comparable levels of variation were found among sub-cultivars (approximately 27%) and among cultivars (approximately 30%). AFLP data and phenotypic observations confirm the high level of variation in commercial seed lots. In order to improve commercial product uniformity, without run into inbreeding depression, the most suitable strategy is the production of F1 hybrid seeds. For this reason a protocol for the production of doubled-haploid lines by anther culture has been set up at the Experimental Institute of Floriculture (Sanremo).

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.58

MICROSPOROGENESIS BEHAVIOUR OF A TRIPLOID LILY

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cytokinesis, Lilium, meiosis, pollen

The genus *Lilium* includes approximately a hundred species classified into seven Sections. This germplasm represents an important source of useful genes as well as a "reservoir" of allelic diversity for breeding purposes. However, gene introgression through the wide interspecific hybridization is hampered by incompatibility barriers at pre- and post-fertilization levels including F_1 hybrid sterility. In this context, meiotic nuclear restitution events, and 2*n*-gametes with various modes of origin can be crucial for breeding strategies based on wide hybridization.

In this work, a screening of *Lilium* genotypes for nuclear restitution events in microsporogenesis was performed through the analysis of chromosome number, pollen stainability and meiosis.

Chromosome number and pollen stainability have been evaluated in *Lilium* L. species (*L. formosanum, L. miryophillum, L. regale*), and in cultivars coming from Asiatic (Elite, Pollyanna, Vivaldi) and Oriental hybrid group (Cascade, Casablanca, Galilei). All the genotypes resulted diploid ($2n=2x=24$) except cv. Elite which was triploid ($2n=3x=36$). The pollen stainability ranged from 90 to 98% among diploids and from 80 to 90% in the triploid which exhibited pollen grains of different size, as well.

A detailed analysis of microsporogenesis was carried out in the triploid cultivar. The chromosome associations at diakinesis were either trivalents or bivalents and univalents. Anaphases I and II exhibited lagging chromosomes and unequal distribution of chromosomes at the two poles. Single and double bridges plus acentric fragments formed possibly due to a paracentric inversion.

Meiotic nuclear restitution mechanisms occurred following the failure of the reductional wall or, alternatively, of the equational wall. The analysis of sporads evidenced tetrads as well as dyads, triads and rare monads and poliads. In conclusion, the identification of restitution mechanisms reinforce the importance of 2*n* gametes for *Lilium* breeding.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.59

ORCHID GERMPLASM COLLECTION, BREEDING AND ASYMBIOTIC GROWTH

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Orchids, breeding

The Floramiata S.p.A. is the biggest Italian company for the production of ornamental plants. One of the most important goals is to create new varieties, to evaluate the Italian wild germplasm and to introduce the selected species in the market.

The orchid breeding programme of tropical and Mediterranean species has been started three years ago, more then 35 botanical species of the genera Phalaenopsis, Cattleya, Dendrobium, Paphiopedium, Phragmipedilum and Phajus have been collected and used for the breeding. During that period several grexes have been produced, currently we are cultivating thousands of hybrids and selecting the most promising new phenotypes.

All the Italian species of the genera Serapias (S. cordigera, S. lingua, S. neglecta, S. nurrica, S. parviflora, S. politisii, S. vomeracea), and many other wild species (Orchis coriofora, Orchis morio, Dactiloryza elata, etc.) have been successfully reproduced by seeds in asymbiotic conditions in vitro and then grown in pots. The results demonstrated that those species could be propagate in industrial manner for the needs of repopulation or as new commercial ornamental plants.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.60

THE PRODUCTION OF ORGANIC SEED

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organic seed production, organic farming

Seed is a strategic component for conventional, organic and Genetically Modified Organism (GMO) based type of agriculture. A strong and efficient seed industry is a pre-requisite for producing quality seed of varieties suitable to maintain the type of agricultural systems chosen by farmers. The total cost of seeds sustained by the Italian farmers, for example, is estimated in about 800 million Euros per year, of these more than 300 million Euros burden our trade balance due to the import of seeds of species such as corn, oilseed crop, vegetables, sugar beet and potato.

In organic farming all the input need be organic, including the seed, and this is regulated by the EU legislation that recommends, beginning from the 1st January 2004, that seed used in organic agriculture must be organic, unless not available in the market.

Important aspects related to the improvement of organic seed production is the control of pests, diseases and weeds. In recent years the concern about research in organic agriculture is increasing. In fact, in 2005 for the first time in Italy it has been funded a project aiming at researching and innovating seed production, particularly for organic farming. The project is promoted by the Regione dell'Umbria, and involves 14 research institutions, scattered in the North, Central and South Italy, including Sicily and Sardinia. Basic themes are: tracing the most suitable areas for seed production, improvement of seed production techniques, finding the most suitable cultivars, the conservation and safeguarding of biodiversity. Forage crops are an important component of the project.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.61

EVOLUTION OF TREES AS DRIVERS OF TERRESTRIAL BIODIVERSITY (EVOLTREE): A NEW EUROPEAN NETWORK OF EXCELLENCE

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network of excellence, genomics, forest trees

The awareness that biological diversity is of fundamental importance for our society has dramatically increased during the last decade since the Convention of Biological Diversity (CBD) was signed in 1993. Diversity is one of the key elements to preserve the adaptive potential and the capacity of organisms to adapt to new environmental conditions. There is a growing concern about the accelerated loss of species, the erosion of diversity under increased human impacts and global change, the modification of the processes shaping diversity. In its broadest sense, biodiversity embraces ecosystem, species and genetic diversity. Within this hierarchy DNA variation is the only reproducible in space and time and offers the possibility for evolution of individuals and species as a response to environmental change. In the past decade genomics has emerged as one of the most sophisticated tool to decipher diversity at its more refined scales (genes and nucleotides). The European Evoltree network aims to apply genomics to understand the past present and future diversity, with a view to regulating its evolution and maintain sustainability. The network aims first to study the genetic diversity of trees, as dominant species and drivers of terrestrial biodiversity, and then to expand its scope to diversity issues at the community level, by considering selected associated organisms of trees, and their interactions with trees. The network intends to capitalise on the substantial expertise and availability of genomic resources accumulated in different countries during the past collaborative projects. It integrates interdisciplinary research (genomics, population and quantitative genetics, ecology, ecophysiology, palaeoecology, reproductive biology, modelling, bioinformatics, conservation biology, silviculture) to decipher the structure, expression and polymorphism of genes of adaptive significance and gain new insight into ecosystem function. The genomic activities are conducted within a 'virtual lab' where high throughput techniques will be developed and then applied to a wide range of tree species, starting with a reduced number of model species. Large data sets will be compiled and made accessible by developing data mining procedures for the analysis of geographic and temporal distribution of genetic diversity. EVOLTREE intends to spread its knowledge and expertise through the development of training capacities and by facilitating mobility opportunities throughout Europe. EVOLTREE is a consortium made up of 24 partners coming from 14 different countries, which has adopted a strong management policy to organise its internal functioning and ensure the durability of the network.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.62

GENETIC STRUCTURE IN *CUPRESSUS SEMPERVIRENS* REVEALED BY NUCLEAR MICROSATELLITES

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population genetics, nuclear microsatellites, cypress

Cupressus sempervirens L. grows in most countries around the Mediterranean basin, where it characterizes the landscape with its special crown shape, and is used for timber production, as a windbreak and for ornamental purposes. Since antiquity, cypress has been extensively cultivated far beyond its natural geographic range, in earlier times through its association with religious rites and later for aesthetic reasons. Recently an effort has been made to identify and characterise new highly polymorphic molecular markers, namely nuclear microsatellites. Nine polymorphic nuclear microsatellites were developed through the construction and screening of an enriched library. The newly developed primers were tested in 38 species of the Cupressus genus. For some SSRs, the transferability rate was higher than 85%. Interestingly, the transferability success rate decreases from Mediterranean-, to Asian-, to Central- and North- American species. Distribution of diversity was analysed in twelve *Cupressus sempervirens* populations, sampled in Italy, and compared with that of some populations from Turkey. Significant departures from panmixia were observed for some loci–population combinations. A strong genetic differentiation among populations (G_{ST}= 0.13) was found, and the amount of differentiation increased with geographic distance. A high proportion of the total variance is due to differences between the two groups of populations (Italy and Turkey). Discussion about the possible origin of the Italian populations as well as about the possible factors originating the deficit of heterozygosity in this species is reported.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.63

NUCLEOTIDE DIVERSITY, LINKAGE DISEQUILIBRIUM AND POPULATION DIFFERENTIATION IN A SET OF CANDIDATE GENES FOR TIME OF BUD SET IN NORWAY SPRUCE (*PICEA ABIES)*

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conifers, adaptation, association study, genetic diversity, linkage disequilibrium

Conifers appear to have sufficient nucleotide diversity and rapid decay of linkage disequilibrium to make allele-phenotype association mapping studies based on candidate genes an attractive approach for the dissection of complex traits. Furthermore the low levels of between population differentiation that were previously observed for neutral markers are expected to significantly alleviate the problem of false positive associations due to population substructuring, one of the major drawbacks in association mapping. In order to evaluate this mapping method based on variation in natural populations we set out to perform an association mapping study in Norway Spruce (*Picea abies*) intended to unveil the genetic factors controlling bud set time and length of seasonal growth. These traits show high heritability in spruce and strong differentiation along latitudinal clines. Candidate genes were chosen among those involved in the light-dependent control of the length of the vegetative season in *Arabidopsis* and other model species. Candidate genes and a set of reference loci were resequenced in seven European populations of Norway spruce distributed over the latitudinal cline observed for bud set. Here we present and discuss the results on the distribution of single nucleotide polymorphisms (SNPs) and the levels of short range linkage disequilibrium observed in the selected loci. Moreover a preliminary analysis of among-populations genetic differentiation will be presented with regard to its potential effects on the association study. The comparison of nucleotide diversity data with phenotypic data may be used to detect significant associations between molecular markers and phenotype and determine the role of the loci analyzed.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.64

DEVELOPMENT OF EST-PCR MARKERS AND SNPS DISCOVERY IN NORWAY SPRUCE (PICEA ABIES (L.) KARST.)

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EST, SNP, Norway spruce, population genetics

A non-normalized cDNA library was constructed from mRNA of 1 month-old bare root Norway spruce seedlings germinated from a population of open-pollinated seeds. cDNA products were cloned into pGEM-3Z (Promega P2151) and 4007 sequences from either the 5' end or the 3' of each clone were obtained. A total of 408 contigs were formed after assembly of the 4007 ESTs. The clusters contained a total of 1324 sequences, whereas 2684 remained as singleton ESTs (67%), not identical to any other EST in the dataset. The clusters represent either independent clones of the same transcript, allelic sequences, or different members of multigene families. The largest cluster contained 36 sequences while 233 clusters contained only 2 sequences. Sequences produced within this project were analysed for homology with already available conifers databases and then partitioned into functional categories. New primers were designed for the amplification of ESTs originating from the cDNA library of Norway spruce and tested together with an additional set of EST primers derived from other conifers (*Pseudotsuga menziesii* and *Pinus taeda*). Parental trees of mapping populations of some conifer species were analysed. In total, 351 EST primers were tested in Norway spruce, among which 54% amplified. Only ESTs showing homology at least between two conifers were selected. Twenty-three Norway spruce ESTs originating from the cDNA library, 8 from *Pseudotsuga menziesii* and 7 from *Pinus taeda* met this requirement. EST polymorphism between the two parental trees of an additional Norway spruce mapping population was detected through sequencing. All amplified fragments of the two parental trees were sequenced from both ends using a capillary MegaBace *Amersham* automatic sequencer. About 50% of the ESTs displayed nucleotide variation between the parental trees, with at least one SNP (Single Nucleotide Polymorphism). The benefits of these newly developed EST markers are outlined with respect to population genetics.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.65

SSCP POLYMORPHISMS ANALYSES OF CATALASE GENE INTRONS FOR CLONAL IDENTIFICATION IN POPLAR

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molecular marker, intron, clonal identification, SSCP, Populus

Conventional clonal identification system, based on combination of morphological and phenological traits, represents a difficult, time consuming and subjective method. Considering the importance of varietal identification in poplar and the necessity to have certificated material to trade, the aim of this work is to develop new molecular markers simple and inexpensive for clonal identification for the breeding and genetic resource management programs.

Preliminary studies, performed in our laboratory, have allowed to develop SSCP (single-strand conformational polymorphism) markers for two poplar catalase genes of *P. deltoides* (*Cat1* and *Cat2*) by designing specific primers flanking introns. Four primer pairs have been selected: two primer pairs (Cat1-A and Cat1-B markers) have been designed based on *Cat1* gene coding sequence spanning intron II and introns V-VI-VII respectively. Two other primer pairs (Cat2-C and Cat2-D markers) have been designed based on *Cat2* gene coding sequence spanning introns II-III and IV-V respectively. In this study, the suitability of developed SSCP markers have been tested by differentiating 96 poplar commercially important clones belonging to *P. alba, P. deltoides, P. nigra, P. trichocarpa* species and largely to *P. x canadensis.*

The results emphasized that SSCP analysis was efficient to detect DNA polymorphism. It is a sensitive analytical tool for interspecific and intraspecific identification. Particularly it could be useful to analyze clones before their registration to RNCF (National Register of Forest Clones) to check material novelty. This type of analysis based on exclusion by similarity with registered clones could represent an economic and temporal saving because it avoids the long valuation procedure for the clones that have no novelty traits.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.66

MULTILOCUS LINKAGE DISEQUILIBRIUM SCAN IN *POPULUS* GENOME

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linkage disequilibrium, poplar genome, association mapping, Populus alba, Populus nigra

Linkage disequilibrium (LD) is currently the focus of many studies for its application in association mapping of quantitative or adaptive traits in natural populations of outcrossing long-lived plants. The extent of LD across the genome under study determines the feasibility of two different association approaches, whole genome scan and candidate gene approach, since it affects the number of markers that should be genotyped.

At the moment, no studies on LD across extensive regions of the genome are available for forest trees. The recent release of the genome sequence of *Populus trichocarpa* offers a unique opportunity to analyse the structure of the poplar genome and map important genes.

Preliminary analysis on sequence variation and linkage disequilibrium in *Populus nigra* showed that LD extends over 5 Kb, but definitely decays within 100 Kb. On the basis of these data, LD has been measured (R^2 measures) at four genomic regions, each 100 Kb in length and composed of different consecutive sequence tracts spaced 1 – 3 Kb apart. These four regions are considered unlinked as distant more than 6 Mb or located on different chromosomes, *i.e.* linkage groups II, VIII, XIV on the *P. trichocarpa* genome. 24 unrelated genotypes of *P. nigra* belonging to a French and an Italian populations and 24 of *Populus alba* belonging to European and Northern Africa populations were analysed in this study. At each genomic tract SNPs were identified and SNP haplotypes determined. We used different softwares (Haplotyper, Phase, LDanalyzer) to infer haplotypes, as a requirement for LD estimation, and we eventually selected LDanalyzer (http://www.chgb.org.cn/lda/lda.htm) after comparison of the haplotype inferences to cloned sequences (known gametic phase).

We first analyzed patterns of LD within the chromosomal regions under study in the two species by considering triangle plots of LD between SNPs (minor allele frequency greater than 0.1), where we observed variable patterns of LD across the four genomic regions surveyed. We next estimated the extent of LD in the two poplar genomes considering the overall LD decay plot for SNP pairs within each chromosomal region. The distribution of R^2 values had a similar trend in the two species with an higher average value in *P. alba* (average R^2= 0.20) than in *P. nigra* (average R^2= 0.14) and in both species the LD slowly decayed to R^2= 0.1 at a distance of 90 kb. Since LD is a property of the natural population under study, we also evaluated LD extent in the two populations (French and Italian) of *P. nigra* considered in this work. The distribution of R^2 values across the four chromosomal regions suggested a slightly more extensive LD in the Italian population than in the French one, even though no significant differences ($P > 0.05$) were detected between the two distributions.

Our results suggest that LD in poplar is not extensive enough for a whole genome association approach, as too many markers should be genotyped for an efficient mapping. On the other hand, the candidate gene approach emerges as a promising approach for association mapping in poplar, since LD extends over distances that span one or a few genes.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.67

GENETIC VARIABILITY IN SICILY POPULATION OF *QUERCUS SUBER*

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genetic differentiation, nuclear microsatellites, Quercus suber, conservation

Sicily is a region with an elevated biodiversity but there is a high risk to lose it in consequence of population reduction and fragmentation of the natural distribution of several forest tree species. This is particular true for those species as *Q. suber* which distribution is fragmentated and restricted to specific areas.

Therefore, it results necessary to investigate how the genetic variability is distributed among and within the populations. Particularly interesting could be to study the situation of this species in the Mediterranean islands as these are particular ecosystems in which conditions as geographic isolation, and genetic drift could influence the genetic variability and therefore the genetic structure of the populations.

The aim of the present study is to investigate the genetic variability of Sicily populations by nuclear microsatellites.

Five Sicily populations distributed from west to east, one Sardinia population, one Tuscany populations, and one France population (Esterel) are analysed. The results on genetic variability are reported and the data are discussed considering the conservation priority for this species.

Preliminary results, obtained by the analyses of two microsatellites, indicates that the allelic pool of Sicily populations is also present in the other populations analysed, and that the genetic haplotipic variability within Sicily populations is comparable to that found in the other populations.

These data indicates that it is extremely important, in future conservation strategies, to maintain the actual genetic variability present within Sicily populations.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.68

GENETIC VARIABILITY AND IDENTIFICATION OF REGIONS OF PROVENANCE IN SCOTS PINE

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Pinus sylvestris, genetic differentiation, genetic variability, propagative material, Regions of Provenance

In recent years, the need of forest seeds has dramatically increased, also due to rules issued by European Community aimed at increasing and improving the forest coverage. In Italy, however, the available seeds are often of poor quality and sometimes their origin is unknown. The need of an optimisation of the forest seed production is therefore clear. At the end of 2003, Italian Government has issued the act no. 386, that implements the European Council Directive 1999/105/CE, concerning the marketing of forest reproductive material. One of the most important feature of the act is the definition of Region of Provenance, that is *"….the area or group of areas subjected to sufficiently uniform ecological conditions in which stands or seed sources showing similar phenotypic or genetic characters are found.…"*.The identification of Regions of Provenance is therefore a basic aspect for a rational management of activities linked with forest trees propagation, including afforestation and *in situ* genetic preservation.

The purpose of this study was the evaluation of neutral DNA markers (microsatellites) as a tool to study genetic variability distribution of Scots pine (*Pinus sylvestris* L.) in Italy, and to group populations according to their genetic similarity. 16 natural ash populations, representing the locations where the species grows in Italy, were sampled and DNA was extracted from young leaves. Six microsatellite primer pairs were used to detect genetic variability. Levels of within and among populations variability were estimated and genetic differentiation was calculated. 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.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.69

GENETIC POLYMORPHISM AT CSN1S1 LOCUS IN TWO CZECH GOAT BREEDS

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casein, goat, polymorphism, biodiversity

White Short-Haired (WSH) and Brown Short-Haired (BSH) are two protected local goat dairy breeds from the Czech Republic. A genetic characterisation of these breeds is needed to allow the preservation and the exploitation of their genetic variability. For this purpose a research project was started with the aim to investigate the genetic structure of the casein gene cluster and to evaluate the haplotype variability among and within populations. In this work we present the first results of this research. The goat CSN1S1 gene represents an excellent model for demonstrating that the major part of the variability observed in the CSN1S1 casein content in the goat milk is due to the presence of autosomal alleles at single structural locus CSN1S1. Genetic polymorphism at the CSN1S1 locus gene is characterized by different alleles which are results of mutation from single substitution, insertion/deletion, interallelic recombination or apparent lack of synthesis of alphas1-casein. So far, this locus is characterized by at least 17 alleles, associated to four different levels of alphas1-casein expression in milk. Alleles A, B, C, H, L and M are associated to a high content alphas1-casein, alleles I and E to an medium content and alleles D, F and G to a low level of alphas1-casein in the milk. Alleles CSN1S1 0_1, 0_2 and N are "null" alleles and have been associated with the absence of alphas1-casein. DNA samples obtained from a total of 498 animals belonging to White Short-Haired (317) and Brown Short-Haired (181), randomly chosen in the flock, were analysed at the CSN1S1 locus. In order to detect carriers of alleles related to a different level of the corresponding milk proteins, DNA samples obtained from a total of 498 animals belonging to White Short-Haired (317) and Brown Short-Haired (181), randomly chosen in the flock, were analysed at the CSN1S1 locus. Genomic analysis was performed by using different molecular techniques (PCR, PCR-RFLP and AS-PCR). Analysis of CSN1S1 locus showed, in both breeds, (WSH and BSH) the prevalence of allele F (0.75; 0.69) which is related to a low level of the protein in the milk. The alleles related with a "high" level of protein synthesis in goat milk are present with a frequency of 0.20 and 0.21, respectively. The null and E alleles were identified with very low frequencies in both breeds. The comparison of these results with those available in the literature showed a similarity with alpine breed characterized by high frequency of allele F. However in alpine breed the E allele is present with a high frequency. Obtained results presented contribute to a better knowledge of the genetic variation at CSN1S1 casein gene in goat breed as gene reserve pool in Czech Republic. Furthermore these first data can be used to monitor the genetic structure of the two breeds to avoid further loss of genetic variability. The research is now extended to the other three casein loci with the aim to study the effects of casein haplotype, instead of individual genotype, on milk production and cheese -making properties of goat milk.

Acknowledgements:
Research supported by the National Agency of Agriculture of the Czech Republic (NAZV) Project n° IG 57051, 2005-2008

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.70

STUDY OF MELANOCORTIN-4 RECEPTOR (*MC4R*) POLYMORPHISM IN ITALIAN LARGE WHITE AND ITALIAN DUROC PIGS: ASSOCIATION WITH CARCASS AND MEAT QUALITY TRAITS

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pig, melanocortin-4 receptor, PCR-RFLP, carcass and meat quality traits

The melanocortin-4 receptor (*MC4R*) plays an important role in the control of mammalian energy homeostasis. In pig this gene, mapped on chromosome 1 (SSC1) shows a single nucleotide polymorphism (G>A) within a *Taq I* restriction site. The alternative alleles resulted in either aspartic acid (GAU) or asparagine (AAU) at position 298 in a region that is highly conserved in the MCR proteins. Kim *et al*., (*Mammalian Genome* 11:131-5; 2000) reported an association between allele A with higher backfat content, higher feed consumption, and faster growing in pigs. By PCR-RFLP the *MC4R* polymorphism was studied in 316 pigs belonging to 11 breeds including Italian Large White, Italian Landrace, Italian Duroc, Belgian Landrace, Hampshire, Pietrain, Meishan, Cinta Senese, Casertana, Calabrese, Nera Siciliana. A preliminary analysis of the relationship between *MC4R* polymorphism and pig production traits was performed. To this aim the allele frequencies between pigs with divergent breeding values (EBV) for backfat thickness (BFT), lean cut (LC), average daily gain (ADG) in Italian Large White breed and for intermuscular visible fat (VIF) in Italian Duroc breed were compared by Fisher exact test. For each trait, 100 pigs were considered, 50 with high and 50 with low EBV values. Significant differences of allele frequencies between divergent Italian Large White pigs were identified for BFT, ADG, and LC. Allele A was the most frequent in pigs with high EBVs for ADG and BFT ($P \leq 0.01$), and in pigs with low values for LC ($P = 0.003$). Moreover the association between the *MC4R* polymorphism with carcass and meat quality traits was further evaluated in a group of 272 Italian Large White pigs by a linear model which included boar and genotype as source of variation. A significant effect of *MC4R* genotype was found. The overall results showed that *MC4R* could be considered a candidate gene associated with production traits in pigs.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.71

ANALYSIS OF ALLELE FREQUENCIES OF THE GROWTH HORMONE RECEPTOR (*GHR*) F279Y MUTATION IN SEVERAL CATTLE BREEDS

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growth hormone receptor, bovine, allele frequencies, DNA polymorphism, QTL

Several studies have indicated that bovine chromosome 20 (BTA20) harbors quantitative trait loci (QTL) for milk yield and composition. A combined linkage and linkage disequilibrium analysis positioned a major QTL in the middle of BTA20 and, for its map position, the growth hormone receptor (*GHR*) gene was considered a strong positional and functional candidate gene, knowing the major role played by the growth hormone axis in the initiation and maintenance of lactation. Analysis of polymorphisms in this gene identified a mutation in exon VIII (a T>A transversion, that causes the replacement of an amino acid, a phenylalanine to tyrosine substitution, in a highly conserved transmembrane domain of the GHR protein at position 279), indicated as F279Y, with highly significant effects mainly on milk yield, protein percentage and fat percentage (Blott *et al.*, Genetics 163:253-266; 2003). Allele F increases the percentage of protein and fat in the milk while has a negative effect on milk yield. On the other hand, allele Y has a positive effect on milk yield and a negative effect on protein and fat percentage. Moreover, these effects seem consistent across populations and breeds.

As a first step in order to further evaluate the effects of the F279Y polymorphism and to investigate its possible use in marker assisted selection (MAS) plans in dairy and dual purpose cattle breeds reared in Italy we studied the distribution of these two alleles at the *GHR* locus in a total of 679 animals (Italian Holstein-Friesian, 108; Italian Brown, 104; Italian Simmental, 104; Jersey, 104; Reggiana, 108; Modenese, 66; Rendena, 85). DNA was extracted from semen, blood, milk or hair bulbs. A new PCR-RFLP method was set up to analyse the T>A point mutation of exon VIII of the *GHR* gene inserting an artificial restriction site by means of a mismatched forward primer. The genotypes obtained from a few animals were confirmed by sequencing of the region containing the polymorphism.

In all breeds investigated allele F was the most frequent and ranged from ~0.95 (Italian Brown and Jersey) to ~0.73 (Italian Holstein-Friesian). In Reggiana and Modenese it was ~0.92 while in Rendena was ~0.82. Frequency of the FF genotype varied between breeds ranging from ~0.89 (Italian Brown and Jersey) to ~0.51 (Italian Holstein-Friesian). Genotype FY was observed with the highest frequency in Italian Holstein-Friesian (~0.43) and the lowest in Reggiana (~0.08). A few animals with genotype YY were identified in Italian Holstein (~0.06), Reggiana and Rendena (~0.04) and Italian Simmental (~0.01). For all breeds no significant deviation from the Hardy-Weinberg equilibrium was observed for this polymorphism.

From these data, it is interesting to note the highest presence of allele Y in the Italian Holstein-Friesian breed compared to the other breeds. This may be due to the fact that this allele, that is suggested to have a positive effect on milk yield, could have been indirectly selected because the selection strategies in this breed have been mainly towards an increase in the level of milk production for many years.

Further studies are needed to confirm the effects of this polymorphism in the investigated breeds and to evaluate its application in MAS programs.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.72

GENETIC VARIABILITY IN *TRIGLA LUCERNA* BY SSR ISOLATION AND mtDNA POLYMORPHISMS IDENTIFICATION

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microsatellite markers, mtDNA, Trigla lucerna

The tube gurnard (*Trigla lucerna L.*) is a teleost species of great commercial importance to European fishing nations bordering the Atlantic and Mediterranean Sea. This species is highly esteemed in many countries on account of its firm white flesh. In order to diversify the production after the rapid increase in the number of fish farms that produce sea-bass and sea-bream, the researchers and operator turned their interest to other valuable marine species like the tube gurnard. The successful performance of *Trigla lucerna* in aquaculture and the lack of knowledge about the genetic population structure has prompted the attention on the evaluation of its genetic variability. Early studies on fishery using molecular genetics focused primarly on the structure of proteins and enzymes. Nowadays research has switched to the study of DNA segments.

Microsatellite markers and mtDNA analysis have been elected to characterise the species. Even though microsatellite isolation is time-consuming and expensive, they are widely used as genetic markers since they are co-dominants, multiallelic, easy to score and highly polymorphic. Microsatellite have never been described neither in tube gurnard fish nor it species from the same suborder. To isolate them from the tube gurnard, the FIASCO (Fast Isolation by AFLP of Sequences COntaining repeats) protocol was chosen. The method, based on a digestion-ligation reaction of the amplified fragment length polymorphism procedure, allowed to isolate a microsatellite loci panel. Further investigations throughout screening of wide population in order to verify the informativity of the microsatellite loci has been accomplishing.

Moreover mtDNA variation has been used in this research because already successfully employed to study the phylogenetic inference and population structure in other teleost. Homologous primers were designed on the armored gurnard (*Satyrichthys amiscus)* fish taking advantage of the interfamiliar relationship, because no useful sequence data were available in the database. The subunit 16S and the cytochrome B mitochondrial gene were studied by direct sequencing. So far alignment of sequences from individuals belonging to different populations harvested interspecific SNPs. More genes sequence will be covered and the analysis will be extended to other populations.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - C.73

PRODUCTION AND MOLECULAR CHARACTERIZATION OF SYNTHETIC POLYPLOIDS OF TUBER-BEARING *SOLANUMS*

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AFLP markers, cluster analysis, polyploidization, potato

Wild *Solanum* species offer unique possibility for potato breeding. They mainly occur at diploid (2n=2x=24) level, and can be easily crossed with haploids (2n=2x=24) extracted from cultivated *S. tuberosum* (2n=4x=48). However, a number of diploid species are sexually isolated and their chromosome complement needs to be doubled before use in crosses with haploids. To introgress resistance to *Phytophthora infestans* into the cultivated gene pool, we have identified resistant genotypes among *in vitro* regenerated tetraploid derivatives obtained from diploid incongruent species *S. bulbocastanum* (blb) and *S. cardiophyllum* (cph). Furthermore, in order to study the genetic variability induced by *in vitro* polyploidization, AFLP analysis was performed on 28 synthetic tretraploids, 10 synthetic diploids, and on the parental genotypes they derived from. The 7 primer-enzyme combinations allowed the detection of polymorphisms of interest between the regenerants with different ploidy level. The genetic material evaluated was clustered based on the matrix of genetic similarities and a dendrogram was constructed to distinguish different genetic groups. The lowest similarity was shown by the diploid parents and their synthetic tetraploids. We hypothize that a combined effect of polyploidization and somaclonal variation has been involved in the observed genetic variability. The implications due to our findings are discussed from a genetic and breeding standpoint. Research ongoing aims to study in details the effect of polyploidization on our synthetic polyploids.