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Poster Abstract – A.01

INNOVATION AND RESEARCH TO SUPPORT THE NATIONAL SEED PLAN

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conventional and organic seed production, GM crops, co-existence, conservation of biodiversity

Innovation and research to support the National seed plan (PRIS2) is an Inter-regional three-year project coordinated by the Department of Applied Biology, University of Perugia, and is aimed at defining the conditions and methods for providing Italian farmers seeds of high quality, necessary to support agriculture in a phase of profound transformation.

The project started in 2005 and involved 6 Italian Universities and 8 Research Institutions, investigated the most important aspects of conventional and organic seed activities. For the most important crops (cereals, vegetable and forages species), suitable production areas, innovations capable of improving the production route, rules to obtain GMO-free seeds were identified. The possible co-existence of conventional and GM crops have been evaluated during the 3 years of the project. A catalogue of annual and perennial species have been created and the most suitable varieties, for organic seed production have been identified. Moreover the techniques for high quality seed production have been defined.

For seeds to be used as a mean for biodiversity conservation, the project has considered several aspects: survey of national and regional programs for germplasm collection, evaluation and conservation; establishment of an inter-regional germplasm conservation network; landrace definition and identification of parameters to be used for landraces registration and commercialization. The activity results of the 3 years will be presented in November 2008 in Perugia during the final conference.

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Poster Abstract – A.02

DETECTION AND QUANTIFICATION OF DIFFERENT POLLEN TAXA BY MEANS OF A REAL-TIME PCR APPROACH

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pollen detection, pollen quantification, Real Time PCR, allergy

A specific, sensitive real-time PCR assay based on TaqMan chemistry was developed for the identification and quantification of air dispersed pollen, in order to provide a rapid and reliable analysis alternative to the traditional and time-consuming microscope based detection methods. Actually, accurate and punctual information of the amount and type of air-dispersed pollen represents a useful tool for the diagnosis and therapy of allergy, moreover it can be essential for monitoring the pollen diffusion of GMOs.

A set of ten pollen taxa was considered for their presence in the local flora and their allergic potential. *Parietaria officinalis*, *Ostrya carpinifolia* and species belonging to *Alnus*, *Betula*, *Artemisia*, *Corylus*, *Fraxinus* genus and *Poaceae* (*Gramineae*) and *Cupressaceae* families were finally tested. For each taxon, pollen and leaves were sampled in three different areas of Trentino region, in order to represent genetic differences within different plant populations.

Bioinformatic analysis was carried out to identify specific DNA sequences for each plant group. The search at NCBI database was focused on single- or low-copy nuclear gene sequences. Performing a BLAST against the non redundant nucleotide database, the specificity of DNA sequences was assessed *in silico*. Amplification of target genomic regions with degenerate primers and sequencing of PCR products were performed when no informative DNA sequences were available such for *Parietaria* and *Poaceae*.

In parallel, an efficient protocol for DNA isolation from both free and sampling strip-immobilized pollen was established. After setting the appropriate real-time PCR conditions, the specificity of primers and probe was verified using DNA isolated either from leaves or pollen.

Specific primer-probe combinations were finally established for the following groups: *Parietaria officinalis, Artemisia annua*, *Betula pendula*, *Alnus, Ostrya carpinifolia, Olea europea*, *Graminacae, Oleaceae* and *Cupressaceae.*

DNA isolated from different pollen species was used for standard curve construction when taxa had to be identified at genus or family level. Preliminary results of quantification analysis of pollen mixtures will be presented.

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Poster Abstract – A.03

IMAGE ANALYSIS MEASURING TOOL TO ASSESS THE MORPHOLOGY AND STRUCTURE OF PLANT CHROMOSOMES

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chromosome, measuring tool, image analysis

A quick and objective imaging method was developed to measure chromosomes after their arrangement in karyograms.

The tool allows the measurement of the morphology of each chromosome independently of their number, size and shape. The centromeric position is drawn interactively by the user together with the satellite position, moreover each chromatid is taken into account in the measurements. Direct measurements of each arm in each chromatid and satellite if any, are stored in a specific data base and ready to use in another application (e.g. Excel electronic table).

If chromosomes have been treated specifically to show patterns (Banding, FISH, GISH, Immunological reaction) it is possible to map on the chromosome each band/spot/paint, and quantify also any of them, i.e. morphological and densitometrical measurements can be easily obtained on specific chromosome paint.

The tool was developed with Zeiss KS-400 V3.0 (Carl Zeiss Vision GmbH, Hallbergmoos, Germany, 2001) image analysis software. It is a versatile image processing program designed to support demanding professional applications, moreover it can be customized for specific applications by editing appropriate image analysis algorithms in ‘‘*Macros’’*, able to automate the analysis.

The tool is freely accessible and open to any collaborative work on plant and animal chromosomes, send us the karyogram photos and we will send back the results.

Karyotyping in plant by an image analysis system 1991. Venora G., Conicella C., Errico A., Saccardo F. J. Genetics & Breeding - 45 : 233-240

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Poster Abstract – A.04

REAL TIME PCR ASSAY TO ASSESS RETROELEMENTS AMOUNT IN *TRITICUM* AND *AEGILOPS* SPECIES AND ITS PHILOGENESIS IMPLICATIONS

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Real Time PCR, retransposons, WIS2-1A, phylogenesis

The assess of variation among related taxa allows geneticists to understand philogenetics relationships and plant breeders to exploit wider pools of diversity. The genetic variation and genetic structure of a species reflects not only the patterns of its genetic exchange, but also its history in term of gene flow, range fragmentation and isolation among population. In present work, we have employed Real-Time PCR technique to analyze the amount of WIS2-1A retrotransposon in some *Triticum* and *Aegilops* species. WIS 2-1A is the first retransposon found in wheat, it was primarily observed as an insertion of 8 Kb into Glu-1 locus, a High-Molecular-Weight (HMW) storage protein gene, in *Triticum aestivum* (AABBDD). WIS 2-1A has a high presence of homologous sequences showing high levels of interspecific variability and almost no intraspecific differentiation, moreover it represents a recently evolved region unique to *Triticeae* genomes. To understand the molecular evolution of this locus used as an index of genetic differentiation, we have compared the amount of retrotransposons WIS2-1A revealed in three ploidy level of wheat: *Triticum monococcum* (*2n= 2x= 14*, AA), *T. dicoccum* (*2n= 4x= 28*, AABB), *T. spelta* (*2n= 6x= 42*, AABBDD), with the corresponding D genome donor, *Aegilops tauschii* (2*n* = 2*x* = 14, DD), B genome donor *Aegilops speltoides* and other two species of *Aegilops (sharonensis and bicornis).* The obtained polymorphism in retrotransposon number has been used to model the temporal sequence of insertion events in a lineage and to establish phylogenetic hypotheses. The obtained results show the presence of WIS2-1A retrotransposon not only in the *Triticum* species examined, but also in the *Aegilops* ones in accord with previous work (Moore *et al*., 1991) where it has been observed that WIS2-1A is a very ancient element present probably in the common ancestors; infact the presence of this retroelement it has been confirmed in all *Triticae* tested (Moore *et al.*, 1991). Moreover, the highest number of retrotransposon was found in *Ae. Speltoides* and *T. spelta*, followed, in decreasing order, by *T. dicoccum, Ae. Tauschii, Ae. Bicornis, Ae. sharonensis* upon the lowest *T. monococcum*. These results confirm previous studies where it has been observed that genome B contain a major number of retrotransposons compared with the D genome.

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Poster Abstract – A.05

IDENTIFICATION OF DURUM WHEAT CULTIVARS BY SSR MARKERS

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durum wheat, cultivar identification, SSR markers

In the Mediterranean countries, Italy plays an important role and long tradition in durum wheat cultivation. Over 170 cultivars of durum wheat are subscribed in the Italian list of variety. Inexpensive and powerful tools are needed in order to assess distinctness, uniformity and stability of cultivars. They would also be useful, a posteriori, to settle legal conflict over the recognition of a seed stock. Generally, morphological traits provide low informativeness, due to limited level of polymorphism, often exhibit a polygenic control and their expression is subjected to environmental effects. Seed protein storage analysis for cultivar identification is limited since protein polymorphism is not so high. A much more powerful and informative tool is provided by molecular markers. Particularly, microsatellites or SSR markers are abundant, multiallelic, highly polymorphic and not influenced by environment.

The aim of this study was: i) to test the efficiency of SSR markers in DNA fingerprinting of durum wheat; ii) to distinguish 80 cultivars with a reduced number of SSR markers; iii) to construct an identification key based on molecular data.

Preliminary, an analysis of SSR markers informativeness in a set of 28 durum wheat cultivars was carried out by using 11 primers. The SSR markers screened included 8 *Xgwm* and 3 *Xwmc*. All primers pairs produced fragments in the examined cultivars. The number of fragments amplified ranged from 4 to 27. The level of informativeness of SSR markers was estimated by Resolving power (Rp) index. The Rp of the 11 SSR markers ranged from 0.90 to 10.14. A strong relationship ($r^2 = 0.90$) was observed between the Rp and cultivar identification. On average, SSR markers with an Rp value of 2.89 were predicted to distinguish 7 cultivars. One SSR marker resulted highly informativeness (Rp = 10.14) and was able to distinguish all 28 cultivars. This SSR marker was tested on 80 durum wheat cultivars and distinguished 69 genotypes. The indistinguishable cultivars were identified by another informative SSR marker. In addition, an identification key was worked out for cultivar identification with the data of two SSR markers.

The present work showed that two primers pairs resulted sufficient to distinguish all durum wheat cultivars examined indicating a very good discriminating ability of SSR markers. This result suggested that the SSR analysis was a technique quick, reproducible and generates several polymorphisms useful in cultivar identification studies.

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Poster Abstract – A.06

LOW MOLECULAR WEIGHT GLUTENIN SUBUNITS IN *TRITICUM TURGIDUM* SSP. *TURANICUM, POLONICUM* AND *CARTHLICUM*

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LMW-GS, tetraploid wheats, two-dimensional electrophoresis, PCR

The low-molecular weight glutenin subunits (LMW-GS) affect viscoelastic properties of dough. They have been subdivided, according to their biochemical properties, into B-, C- and D-types: the B types are typical LMW-GS, the C- and D-types correspond to modified monomeric proteins, similar to the so called gliadins. The LMW-GSs, mainly encoded by a multigene family present at the *Glu-3* loci located on the short arms of the group 1 chromosomes closely linked to the *Gli-1* loci, have not been completely isolated and characterized in tetraploid wheat.

In order to study the polymorphism of these proteins, seventy-five accessions of *T. turgidum* ssp. *turanicum*, ssp. *polonicum*, ssp. *carthlicum*, previously selected by electrophoretic (1DE) and chromatographic (RP-HPLC) techniques, have been evaluated by two-dimensional electrophoresis (2DE) and polymerase chain reaction (PCR). Comparison of 2DE and PCR results have permitted the identification of B- and C-type LMW-GSs and of unknown putative C-type LMW-GSs in the tetraploid species and the designation of corresponding genes.

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Poster Abstract – A.07

RECOGNITION OF GENOTYPES IN DURUM WHEAT SEMOLINA MIXTURE BY AFLP IN FLUORESCENCE

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fAFLP, genotype identification, semolina mixture, durum wheat

The DNA extracted from semolina of sixteen Italian durum wheat cultivars were analyzed by in-fluorescence Amplified Fragment Length Polymorphism (fAFLP) in order to obtain the characteristic fingerprintings of genotypes. The aim of this work was to analyze and recognize durum wheat genotypes in semolina mixture.

All sixteen varieties of *Triticum durum* and their semolina mixture were tested with 6 primer combinations, previously selected as inducing the highest polymorphism levels as well as a uniform distribution of peaks in the region analyzed (50 - 600 bp), to evaluate the effectiveness of fAFLP tecnique in revealing the presence of known genotypes and the method sensibility. Amplified fragments were analyzed by capillary electrophoresis with genetic analysis system CEQ8000^{TM} by Beckman & Coulter, according to Beckman-Coulter Protocol (A-2015A, 2005). By using this fAFLP methodology a DNA fingerprinting of each durum wheat cultivar was generated for genotype identification.

In semolina mixture, obtained data revealed some missing peaks which were present as polymorphic marker in each varieties.

The research is in progress in order to clarify which devices imply the loss of polymorphic markers, which allow the recognition of varieties from semolina when they are mixed too, in order to modify and improve the extraction protocol to obtain the expected fAFLP profiles.

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Poster Abstract – A.08

IDENTIFICATION OF A MAJOR QTL ON CHROMOSOME ARM 7BL FOR DURABLE LEAF RUST RESISTANCE IN DURUM WHEAT

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leaf rust, durum wheat, durable resistance, seedling resistance, adult plant resistance

Leaf rust is a threat to durum wheat (*Triticum turgidum* L. var. *durum*) production. However, genetic and molecular mapping studies aimed at characterizing leaf rust resistance genes in durum wheat have been undertaken only recently.

In this research, we targeted the genetics of the resistance to leaf rust (*Puccinia triticina* Eriks.) conferred by Creso, an Italian durum wheat cultivar released in 1974 from CIMMYT's and Italian materials. This resistance can be considered as durable having been effective since 1975 in a wide range of environments.

The genetic basis of leaf rust resistance was studied using 176 recombinant inbred lines (RILs) from Colosseo x Lloyd and 62 advanced lines derived from multiple crosses involving Creso or its resistant derivatives in their pedigree. RILs were tested under field conditions with a mixture of Italian leaf rust isolates and at the seedling stage with single isolates. In the field experiment, the percentage of infected leaf area was evaluated at three stages through the disease developmental cycle and the area under disease progress curve (AUDPC) was then calculated. A major QTL (*QLr.ubo-7B.2*) for leaf rust resistance at both adult (field conditions) and seedling stages was identified on the distal region of chr. 7BL. In the field, the QTL showed an R^2 of 72.9% and a peak LOD score of 44.5 for AUDPC. The presence of this major QTL was validated by a linkage disequilibrium-based test using field data of advanced lines from multiple crosses. The association results confirmed the QTL location between *Xbarc340.2* and *Xgwm344.2*, with the corresponding AUDPC R^2 values ranging from ca. 10 to ca. 35% depending on the year. *QLr.ubo-7B.2* maps in a gene-dense region (7BL10-0.78-1.00) known to carry several genes/QTLs in wheat and barley for resistance to rusts and other major cereal fungal diseases, including *Lr14a* and *Lr19*, two major candidates for this gene. The availability of precise genetic stocks for the above genes/QTLs in a homogeneous genetic background will facilitate gene postulation studies and, eventually, positional cloning of new resistance genes.

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Poster Abstract – A.09

GENETIC ANALYSIS OF *SOIL-BORNE CEREAL MOSAIC VIRUS* (SBCMV) RESISTANCE IN A DURUM WHEAT MAPPING POPULATION

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resistance QTL, plant disease, durum wheat, SBCMV tolerance

Soil-borne cereal mosaic virus (SBCMV) is a Furovirus responsible for an important mosaic disease of wheat widespread in many wheat growing areas. Most of the durum wheat cultivars show a susceptible to medium-resistant response. Nevertheless, valuable sources of resistance have been identified (Rubies et al., 2006. Proc. 12th Congress of the Medit. Phytopat. Union, Rhodes, Greece, 10-15 June, pp. 100-102; Ratti et al., 2006. Plant Dis Protection 113: 145-149).

Recently, a main locus for disease resistance (*Sbm1*) has been identified in hexaploid wheat on chromosome 5DL (Bass et al., 2006. Genome 49: 1140-1148), but this resistance is not readily available to durum wheat breeders, due to its location on the D genome.

The objective of this study was to map a valuable source of SBCMV resistance using a durum population of recombinant inbred lines (RILs).

Genetic analysis of SBCMV resistance in durum wheat was carried out using a population of 181 RILs obtained from Meridiano (moderately resistant) x Claudio (moderately susceptible). The RILs were characterized for SBCMV response in the field under severe and uniform SBCMV infection during 2007 and 2008 and profiled with SSR and DArT markers. A wide range of disease reaction (as estimated by symptoms and DAS-ELISA) was observed. Most of the variability for SBCMV-response was explained by a major QTL (*QSbm.ubo-2BS*) located in the distal telomeric region of chromosome 2BS near *Xwmc243*, with the favourable allele contributed by Meridiano. QTLs with minor effects on SBCMV-response were also detected. Consistently with the observed transgressive segregation, both parents contributed resistance alleles. *QSbm.ubo-2BS* significantly affected grain yield and test weight of the grains.

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Poster Abstract – A.10

FINDING A MOLECULAR MARKERS LINKED TO THE DEHYDRATION RESPONSIVE TRANSCRIPTION FACTOR 1 GENE (*TdDRF1)*

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Triticum durum, Dehydration Responsive Transcription Factor 1 (TdDRF1), Simple Sequence Repeat (SSR), Random Amplified Polymorphic DNA (RAPD)

The *Triticum durum TdDRF1* gene encodes for dehydration responsive transcription factors. The expression profile of this gene is being evaluated in both greenhouse and field environments, under well-watered and dehydrated conditions. Moreover, we are getting more and more information about the function of these factors. All the data obtained spotlight that the targeting of this gene could help the selection of cultivars for improving the plant tolerance to drought.

For this reason, one effective goal is to find some *TdDRF1* gene-associated molecular markers. Actually, molecular markers are becoming a powerful tool for crop improvement, permitting to characterize and evaluate the allelic diversity of germplasms.

Our analyses was lead on sequences coming from several Italian durum wheat varieties of economical interest for pasta production, some CIMMYT released cultivars with good adaptation to rainfed environments and some durum wheat wild ancestors, *Triticum urartu* and *Aegilops speltoides*.

In particular, here we focused on a Simple Sequence Repeat (SSR), which is localized in the 5' terminal codifying region of the gene and presents a repeating unit of 3 bp in length encoding for an Alanine. Among the analyzed sequences some of them showed a stretch of seven Ala, while others a stretch of six.

In parallel, we used an approach derived from the Random Amplified Polymorphic DNA (RAPDs) technique to screen the *TdDRF1* gene in several genotypes. We selected some specific primers and put at point a multiplex reaction in order to obtain a genotype-dependent band profile. We are performing this PCR assay using both genomic DNA and cDNA, proceeding from stressed plants.

Both the worklines would allow us to compare and cluster the analyzed genotypes based on the similarities concerning the SSR region and the fingerprints of the PCR results.

Hopefully, in the future, we would be able to associate the particular marker allele to the phenotypic response of the wheat genotypes to dehydration conditions.

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Poster Abstract – A.11

GENETIC DIVERSITY AND INTROGRESSION IN MAIZE LANDRACES FROM CENTRAL ITALY

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landrace, SSRs, introgression, selection, in situ conservation

In Europe, flint maize landraces are still cultivated, particularly in marginal areas where traditional farming is often practised. In Italy, their cultivation is generally linked to the production of 'polenta', for which dent corn is not suitable from a quality point of view. We have studied the evolution of maize landraces from central Italy over 50 years of *on-farm* cultivation, when dent hybrid varieties were introduced and their use was widespread. We have compared an 'old' collection, conducted during the 1950s before the introduction of hybrids, with a recent collection of maize landraces. We have included a collection of maize landraces from northern Italy, flint and dent hybrids and inbred lines as controls. 21 microsatellites and 170 AFLP molecular markers were used. Our results show that the maize landraces collected in the last 5-10 years have evolved directly from the flint landrace gene pool cultivated in central Italy before the introduction of modern hybrids. The population structure, diversity and linkage disequilibrium analyses show a significant amount of introgression from hybrid varieties into the recent landrace collection. However, the recent landraces did not show genetic erosion, despite the drastic reduction in the cultivation of maize landraces after the introduction of the maize hybrids. This result suggests that *in-situ* conservation of landraces is an efficient strategy for the preservation of genetic diversity. Finally, the level of introgression detected was very variable among the recent accessions (farmer's fields), with most of them (58%) showing a very low level of introgression. This suggests that co-existence between different types of agriculture is possible, with the adoption of more correct practices that are aimed at avoiding introgression from undesired genetic sources.

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Poster Abstract – A.12

SEED CALORIFIC VALUE IN DIFFERENT MAIZE GENOTYPES AND CORRELATION ANALYSIS WITH SOME SEED CHARACTERISTICS

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maize seed, calorific value, oil content, percent embryo, seed weight

The extensive use of fossil fuels implies a number of problems worldwide. On the environmental side, CO_2 emissions from fossil fuels are believed to contribute to global climate change. In this context, most countries have issued energy and environmental policies that stresses the use of biomasses. In fact, biomasses represent an important source of renewable energy with the potential to diminish both dependence on fossil fuels and CO_2 emissions. Useful biomasses are animal waste, urban solid waste, but principally vegetal biomasses, including whole plants, plant products, plant residues and wastes. Energy from biomasses can be exploited in several ways: direct burning to produce heat or electrical energy (e.g. dry biomasses: wood chips, pellets, etc.), as transport fuel (e.g. biodiesel, etc.), or as chemical feedstock for fermentation (e.g. bioethanol, biogas, etc.).

The exploitation of biomass for energy arises problems when its production comes in competition with that of food crops. However, a moderate use of a food crop, such as maize, for energy production can still be beneficial in some circumstances. Direct burning of mycotoxins contaminated maize grain for house heating or for harvested grain drying may represent an interesting and economically proficient way to salvage otherwise unusable production.

The aim of this work is to find those seed parameters correlated with the heat content, which can be helpful to assess the suitability for direct burning of existing maize genotypes. Such parameters could also be useful in breeding programs aimed at seed calorific value. The genotypes included in this study are: four commercial hybrids (PR 33A46, DK 6530, NK HELEN, DK 440), the B73xMo17 hybrid, the Scagliolo population, two inbred lines (B73 and Mo17), a pop corn variety, a high oil genotype and a sugary mutant.

Since the heat obtained by direct burning of biomass depends mostly on its gross calorific value and moisture content, in this communication we present the gross calorific value of the genotypes analyzed. In addition, our results indicates that seed weight and oil content show a significant correlation with calorific value. In particular, we concluded that seed weight seems a good parameter to asses suitability for direct burning of seeds of most maize genotypes.

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Poster Abstract – A.13

A NEW MUTATION OF DWARF8 MAIZE GENE

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maize, dwarf mutants, gibberellins, DELLA domain

The large increase in rice and wheat yields during the years of the so-called ''Green revolution'' largely resulted from the introduction of monogenic dwarfing traits into plants in combination with the application of large amounts of pesticides and fertilizer. Height reduction has been associated with increases in yield in several different crop species. The new varieties used today are shorter, more resistant to storm damage and have an increased grain yield in comparison with the older ones. The reduction in size of these varieties is caused by the abnormal response to the gibberellins (GAs), which are essential endogenous regulators of plant growth, suggesting that this hormone is central to the control of plant stature. Much of the current progress with regard to GA metabolism comes from the isolation and characterization of single-gene mutants.

However, the elongation of plant organs is a complex phenomenon mediated by many plant hormones, including auxins and brassinosteroids as well as the gibberellins. More than 20 independent dwarf mutants have been mapped in maize altogether representing a very heterogeneous category of dwarfing mutations.

In this work we have isolated a new dwarf mutant that arose in a F1 maize population. It is characterized by reduced stature, due to shortening of internodes rather than reduction of internodes number, and dark green, crinkly leaves. Mutant plants exhibit ectopic anthers in the ear and show variation in the flowering time. Based on morphology and on response to treatments with GA3 the mutant was classified as dwarf with little response to gibberellic acid.

The genetic analysis performed to understand the inheritance of this mutation, named *D*1023*, demonstrated a monogenic dominant inheritance of this trait.

Furthermore, using SSRs (Simple Sequence Repeats) molecular markers on a segregating F_2 population it has been established the mapping position of *D*1023*.

The results obtained from this analysis showed that *D*1023* maps on chromosome 1 (bin 1.09), where *D8-ref*. was localized. Expression analysis carried out using RT-PCR did not show any significant difference between the level of expression of *D8* gene in mutant and wild type.

The novel mutant allele was cloned and the alignment with *d8*(+) wild type alleles present in the database has shown the molecular lesion: an insertion of 3bp within VHYNP domain, localized in the 5' of the gene, near the DELLA domain, responsible for the GA response.

Further data will be presented to better characterize this new mutation.

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Poster Abstract – A.14

AUXIN ROADS AND PLANT ARCHITECTURE: ZmPIN1 PROTEIN LOCALIZATION STUDIES IN MAIZE

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PIN-formed1, polar auxin transport, localization studies, GFP fusion, Zea mays

Modifications in plant architecture have been crucial to the domestication of wild species. For example the domestication of maize from the wild grass teosinte was accompanied by major morphological modifications in both vegetative and reproductive structures. In particular the architecture of the shoot system affects the plant's light harvesting potential, the synchrony of flowering and seed set, and finally the reproductive success.

The plant hormone auxin regulates a wide variety of processes, including embryogenesis, all type of organogenesis, vascular tissue differentiation, root meristem maintenance, root elongation, apical dominance and tropic growth responses to environmental stimuli. In particular polar auxin transport (PAT) is implied in lateral organ initiation at the SAM, where it determines the position of flowers and leaves around the inflorescence stem. This transport is established and maintained by the member of two gene families: the *PIN* and *AUX/LAX* families. It has been shown that the polar localization of PIN auxin efflux carriers correlates with the direction of auxin transport and with the local accumulation of auxin in adjacent cells, suggesting that PIN polarity drives the direction of intercellular auxin flow. In addition the polar localization of PIN auxin efflux carriers changes in response to developmental and external cues in order to channel auxin flow in a regulated manner for organized growth.

In *Arabidopsis thaliana* several genes regulating the polar targeting of PIN proteins have been identified: AtPIN1 basal localization is mediated by the GNOM ADP ribosylation factor/ guanine nucleotide exchange factor (ARF/GEF) that functions in endosomal vesicle formation, while PINOID, a serine/threonine protein kinase, controls the polarity of PIN localization by direct phosphorylation of specific PIN residues. Furthermore, PIN protein sequence itself contribute to the control of polar PIN polarization thank to the presence of sequence-specific signals. Auxin itself also modulates the expression of subcellular localization of PIN proteins, contributing to a complex pattern of feedback regulation.

During our studies on the role of maize *PIN1* orthologous genes during endosperm and embryo development we observed different ZmPIN1 proteins localization pattern in different tissues. ZmPIN1 proteins mark, without any evident polarization, all the cell plasma-membrane of the endosperm transfer cell layer, while, in the embryo-surrounding region, ZmPIN1 proteins are exclusively localized in cytoplasmatic endo-vesicle. On the contrary, ZmPIN1 proteins are polarly localized in the embryonic root, hypocotyl and at the level of the SAM where a new leaf primordium is formed. To better understand the mechanisms underlying plasma-membrane insertion of ZmPIN1 proteins we analyzed the cell membrane targeting ZmPIN1::GFP fusion constructs in homologous and heterologous systems by maize and tobacco protoplast transient transformation and tobacco leaf agroinfiltration. In addition a maize ZmPIN1a::YFP reporter line is

under investigation. Preliminary results let us hypothesize that the three ZmPIN1 proteins may have different plasma-membrane insertion ability and may be subjected to different regulation pathways, in order to allow specific pattern of tissues and organ differentiation.

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Poster Abstract – A.15

FINE MAPPING OF TWO QTL FOR HETEROSIS IN MAIZE

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heterosis, QTL, maize, residual heterozygous line, near isogenic line

Although heterosis is widely exploited for crop improvement and breeding, a clear understanding of its genetic bases is still elusive. In a previous work undertaken to shed light on the genetic basis of heterosis in maize (*Zea mays* L.), we applied a joint classical genetic and QTL analysis to a population of Recombinant Inbred Lines (RIL-$F_{12:13}$) originated from the single cross B73 x H99. Level of heterosis for several agronomic traits and underlying genetic effects were evaluated, together with the relationship between level of heterozygosity and phenotypic performance, and several QTL with heterotic effects on phenotypes were detected. Based on these findings, we followed an introgression program employing marker-assisted breeding on residual heterozygous lines (RHL) to produce pairs of NILs homozygous either for one or the other parental inbred allele (i.e. B73 or H99) at the selected heterotic QTL regions. Large F_2 populations, each segregating only for the respective QTL region, were produced from F_1 hybrids obtained by crossing contrasting NILs for each QTL.

In this work we describe the results of our research work aimed at the fine mapping of two of the introgressed QTL mapped on chromosome 4 (bin 10) and 10 (bin 03). In particular, 3840 and 1152 F_2 plants were genotyped at markers flanking QTL 4.10 and QTL 10.03 respectively, and F_3 families were produced by selfing F_2 plants recombinant at the respective QTL region. The so obtained F_3 families are highly-informative and will be evaluated for agronomic traits in order to fine map their respective QTL.

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Poster Abstract – A.16

GENETIC DIVERSITY AND CLINE OF VARIATION IN BARLEY LANDRACES FROM THE CENTRAL HIGHLANDS OF ETHIOPIA

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Hordeum vulgare, selection, cline of variation, autocorrelation, genetic landscape

Ethiopia is a secondary centre of diversity for barley (*Hordeum vulgare* L.), where barley is the third most important cereal crop. In several regions of Ethiopia, barley is often grown in two different planting seasons per year: during the long rainy season (*Meher*), and during the short rainy season (*Belg*). To determine for the first time the role of this 'two-season-system' on the structure of the genetic diversity of barley landraces, we performed an analysis of a hierarchical collection of barley (seasons, districts within seasons, altitude classes within districts) from North Shewa, in the central Highlands of Ethiopia. Overall, 106 landrace populations were analysed, using both morphological (8 traits, 3,170 genotypes) and molecular (7 SSR, 212 genotypes) markers. The divergence between the populations collected in the *Meher* and *Belg* seasons was very limited. The genetic variation was ascribed to differences between altitude classes rather than between seasons or among districts. The altitude largely overrides geographical distance as the main cause of divergence among individual genotypes. These results are discussed particularly in the context of the exploitation of these landraces for plant breeding and genetic analysis.

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Poster Abstract – A.17

YIELD PERFORMANCE OF NAKED BARLEY NILs AND QTL ANALYSIS OF YIELD TRAITS IN A NAKED X HULLED DH POPULATION

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barley, NIL, DH, QTL analyisis, yield components

In order to determine the physiological effects of the naked/hulled caryopsis character upon barley grain yield components, a NIL population (32 lines BC5F2) developed by backcrossing five times the naked cultivar 'Iabo' (donor parent) to the hulled cultivar 'Arda' (recurrent parent) was employed. Naked NILs, that possess the same (about 80%) genetic background respect to the cultivar 'Arda', but only differ at the locus controlling the kernel type character, were developed as reference lines to remove the interaction that the other genetic background of the naked cultivar 'Iabo' might have on yield. In this context we conducted a comparison and systematic evaluation of agronomic performances and yield components among these NILs and their hulled counterpart, together with recurrent and donor parents in replicated yield trials, at Fiorenzuola d'Arda (Italy), in two years (2005 and 2006).

The naked near-isogenic lines resulted to have the potential to return an equal grain yield compared to hulled genotypes only if they were adjusted by the weight loss of the hull. The interaction between the naked/hulled trait and the different yield components has been studied, and results are here presented.

To further analyze the effect of the *nud* gene, (located on chromosome 7H), in a different genetic background by means of a genetic (QTL) analysis, we analysed data of grain yield and other agronomical traits in a barley doubled haploid (DH) population, derived from the cross 'Proctor' (hulled) x 'Nudinka' (naked). A total of 101 PN lines were evaluated in 1998 for yield, plant architecture, seed weight, biotic and abiotic resistances in yield replicated trials in four contrasting environments in Italy. The software MapQTL v5.0 was used to determine the genomic regions (QTL) controlling the described traits and the overall QTL picture is here presented and discussed.

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Poster Abstract – A.18

GENOTYPING A COLLECTION OF ITALIAN AND EXOTIC TEMPERATE RICE GERMPLASM

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Oryza sativa, molecular marker, phylogenetic analysis, varietal identification

The study of the genetic diversity and populations structure may represent a powerful tool for breeding programmes of crop plants. The association between the visual observation (phenotyping) in the field and the modern bio-molecular analyses carried out by means of genomic markers (genotyping) can be used for the identification of new alleles for elite characters.

The genomic relationship among a collection of 224 rice genotypes was investigated using the microsatellites molecular markers: these are codominant, easy to analyze and allow a high automation and repeatability of the analyses.

A panel of 24 SSR molecular markers was chosen to investigate the rice collection and the genetic distances were analyzed: a phylogenetic tree describing the genetic structure of the collection was created. The correlation genotype-phenotype reveals the existence of distinct phylogenetic groups that were recognisable for their origin and agronomic traits, thus allowing the identification of well-defined groups of cultivars with specific characters.

Moreover, the use of molecular markers are of major interest in the varietal identification in view of traceability, certification of DOP and IGP labelled product etc. and the identification of a given variety classified into a class with a brand name, is allowed. DNA-based analysis with the development of variety-specific molecular markers allows a rapid identification of a product and its attribution to a defined variety within a specific group. We have developed a set of molecular markers (SSR) that can be used as tool for varietal identification, suitable for use directly on grain and on rice-derived products. Fingerprinting analyses were validated on leaf-derived DNA and on flour-derived DNA in commercial varieties, and the developed markers will be exploited as a powerful tool for genetic traceability of the Italian rice.

This study acknowledges the financial support of the project VALORYZA (D.M. 301/7303/06, date 05.12.2006, MIPAAF) and project Riceimmunity funded by Fondazione Cariplo to PTP, Lodi.

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Poster Abstract – A.19

DEVELOPMENT OF MOLECULAR MARKERS FOR THE INTROGRESSION OF BROAD SPECTRUM BLAST RESISTANCE GENES INTO ITALIAN RICE GERMPLASM

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rice, rice blast resistance, PCR-based marker, gene pyramiding

Rice blast, caused by the fungus *Pyricularia grisea* (sexual form *Magnaporthe grisea*), is the most economically important fungal disease in the world's rice-growing areas. Development of resistant cultivars is considered the most effective method to contrast the pathogen. However, cultivars undergo rapid breackdown in their resistance mainly due to the emergence of new pathotypes, due to the high level of instability in the genome of the pathogen (Kiyosawa, 1972; Bonman et al., 1992). More than 40 rice blast resistance genes have been identified and most them have been mapped on the rice genome. In the breeding strategy, the possibility of pyramiding two or more resistance genes into a genotype, is considered a powerful tool to build up broad and durable resistance in a new variety (Francia et al., 2005). Italian rice germplasm is generally characterized by moderate to high sensitivity to blast; in particular, no known blast resistance genes have been introgressed into target varieties and this results in a generalized susceptibility of the cultivated Italian rice germplasm to the pathogen. Molecular assisted breeding is therefore required as major tool to build up new competitive varieties.

In the present work, we have collected a series of 25 rice genotypes bearing 13 known broad range resistance genes, effective against blast. More than twenty-five PCR-based molecular markers linked to these genes have been developed from published primers or by designing primers in genomic regions tightly associated to the genomic map position of the selected genes. Allelic variation of the molecular markers obtained (SSR, CAPS, STS, InDel) was evaluated into the donors of the blast resistance genes and within a representative collection of about 90 rice genotypes, including traditional and modern rice varieties, and varieties for special use. Polymorphic combinations allowing both the introgression of the broad spectrum resistances into susceptible genetic background and the pyramiding of resistance genes, have been identified, thus confirming the potential of the identified markers for molecular-assisted breeding. Molecular analyses and genotyping of the rice collection are coupled to pathogenicity assays performed on seedlings in controlled conditions, with three known blast isolates representative of the Italian blast population, and in field conditions to verify the efficacy of the known resistance genes against the actual pathogen pathotypes.

This study acknowledges financial support of the project CEREALAB (Regione Emilia Romagna) and of the EU co-funded project EURIGEN (049 AGRI GEN RES).

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Poster Abstract – A.20

FIELD EVALUATION OF TEMPERATE RICE GERMPLASM FOR BLAST RESISTANCE

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blast, Pyricularia grisea, temperate rice germplasm, disease evaluation

Blast disease, caused by the fungal pathogen *Pyricularia grisea* (Cooke) Sacc. is one of the most important biotic stress in rice (*Oryza sativa* L.). Blast affects both temperate and tropical rice varieties grown under different environmental conditions, showing higher rates of infection in areas where high-input rice cultivation is practiced. Development of resistant varieties and utilization of chemical fungicides are considered to be the most effectives methods against blast disease. Italian rice germplasm, including old traditional and modern varieties, is characterized by a medium-high level of blast susceptibility. The identification of genetic sources of broad spectrum and durable resistance in the temperate and exotic rice germplasm collection, is of major importance in the breeding programmes in view of identifying valuable parentals in crosses.

Field experiments are conducted in order to evaluate blast resistance of different temperate rice genetics resources at CRA-RIS in Vercelli. In year 2007, a total of 112 rice varieties (Italian rice varieties, exotic varieties and advanced CRA lines) were grown in a field nursery assembled in plots where natural infection was obtained with alternate rows of cv. Maratelli (susceptible check and infection source) and tested genotypes. The rice varieties were sowed in miniplots (1.5 m long and 0.12 m row spacing) with 8 rows (one row for each variety). Rice was drill seeded at approximately 5 g of seed for each row, in dry soil conditions. Permanent flooding was established at 3-4° leaves development stage and the soil was then kept submerged until 1 month before harvest. A total of 300 kg N/ha (supplied by urea fertilizer) were distributed into soil, to enhance susceptibility and create the proper environment for disease spreading.

The disease scores were visually investigated using a 1-6 infection scale according to international protocols, where 1 indicated no blast lesions and 6 indicated high susceptibility. Data were collected one time during the growing season, at physiological maturity stage, when the susceptible reference variety was totally destroyed by fungus. The collection was also tested with direct infection of 3-4 leaf stage seedlings by three selected blast strains (It2, It3, and It 10), representative for pathogenicity in the Mediterranean area.

Results showed a considerable variation among the rice varieties for disease reaction. Only two rice varieties scored value 1 of the infection scale: the Chinese *indica* variety TeQing (blast resistance gene *Pi-b*) and advanced CRA line ISC597. Value 2 of the infection scale was assigned only to 25 rice varieties. Twenty nine varieties scored value 3, 12 varieties rated 4. The high susceptibility to blast disease (values 5 and 6 of the infection scale) was assigned to 44 rice accessions. The *Pyricularia* field experiment revealed the existence of rice genetics resources with high resistance score for the natural pathotypes occurring, thus providing valuable data on genetic material to be used into breeding program. Molecular analyses of the collection are also underway

in order to genotypize the rice germplasm available for known resistance genes (genes *Pi*) by means of molecular markers.

This work is performed within the framework of the EU project EURIGEN (049 AGRI GEN RES) and the project VALORYZA financially supported by Italian MIPAAF (D.M. 301/7303/06).

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Poster Abstract – A.21

MORPHOLOGICAL CHARACTERIZATION OF *ARABIDOPSIS THALIANA* (L.) HEYNH. ECOTYPES COLLECTED IN ITALY USING AN IMAGE ANALYSIS SYSTEM

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Arabidopsis thaliana ecotypes, morphological characterization, model plant

The model plant *Arabidopsis thaliana* (L.) Heynh., a small annual flowered species of Brassicaceae family, shows a wide range of genetic and morphological variation among naturally occurring populations collected in the field. It has a worldwide distribution and can be found in different habitats, for instance in open or disturbed habitats, on sandy soils or on riverbanks, at the sea level or at high altitude, up to 4000 m a.s.l.. Many different genetic systems are involved in the observed plasticity of this species, therefore the wild populations represent the base material to study how different phenotypes are determined and which genes/genetic mechanisms are involved in adaptation.

Considering that this species has been poorly collected from locations in Italy and in the Mediterranean Basin, at the Institute of Plant Genetics (IGV) of CNR (National Research Council) of Bari (Italy) a research activity was started on collection, conservation and morphological characterization of *A. thaliana* ecotypes from Italy. Morphological characters for plant description were based on the Descriptors for Rocket (IPGRI 1999). They were recorded both traditionally and by means of a KS-400 image analysis system. This is a versatile image processing system that can be customized for specific applications by editing appropriate algorithms in "macros", and it has been successfully used in species identification through shape, dimension and texture measurements of whole plants or leaves. Image analysis allowed to create an archive of morphometric data for all ecotypes; the statistical processing, obtained through LDA (Linear Discriminant Analysis Method) of the whole archived data, has allowed the creation of a classifier for the discrimination of germplasm collection with a performances of classification from 83.3% to 100.0% for each individual of a population. Moreover, morphological data gathered by traditional methods were subjected to cluster analysis in order to test the discriminant effectiveness of the selected characters. Both methods allowed a precise discrimination of samples and these results support the effectiveness of the selected characters in describing collections of *A. thaliana*.

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Poster Abstract – A.22

EARLY STEPS INTO SUNFLOWER TILLING

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sunflower, TILLING population, fatty acids

Cultivated sunflower (*Helianthus annus* L.) is one of the four most important annual oil seed crops in the world. Sunflower kernels produce a valuable edible oil rich of unsaturated fatty acids like oleic and linoleic, but it should also been considered as an important crop for biodiesel production, particularly in southern European countries.

The manipulation of fatty acid composition, by means of genetic tools, allows to obtain a product useful for nutritional or industrial purposes (high-oleic mutant line obtained by chemical mutagenesis).

To this aim, TILLING (Targeting Induced Local Lesions IN Genomes) (Comai *et al.,* 2006) represents a powerful tool to identify novel genetic variation in genes that affect key traits.

Seeds of an inbred line of sunflower were treated with four EMS concentrations for different times (6 and 3 hours) in order to establish the acceptable percentage of germination after the treatment. The best results for the germinability test (0.7% EMS) was chosen for the treatment. Mutagenized seeds have been grown to obtain 4211 M_1 plants. To avoid ambiguities caused by chimerism of mutant plants in the first (M_1) generation, M_1 plants were self-fertilized, and M_2 progeny from single seed descent was used for screening. 3899 M_2 plants were regularly observed and screened regarding visible and interesting mutant phenotypes.

For the initial set up of TILLING procedure, two SNP markers were used; different *Cel*I concentrations (1:2, 1:4, 1:10 and 1:20 dilutions) and digestion times (15, 30 and 45 min) and different DNAs pooling (2-4-6-8-12-16 fold) were tested.

Heteroduplex analysis for mutation detection has been adapted to sunflower seeds. The DNAs from 771 M_2 plants are going to be analysed for a pilot screen on three genes (3-keto-acyl-ACP synthase II, oxoacyl synthase and acetyl CoA carboxylase).

Comai *et al*., 2006. TILLING: practical single nucleotide mutation discovery. *The Plant Journal* 45: 684-694

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Poster Abstract – A.23

GENETIC CHARACTERIZATION OF SICILIAN FENNEL LANDRACES BY SSR MOLECULAR MARKERS

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genetic diversity, molecular markers, simple sequence repeat

Fennel (*Foeniculum vulgare* Mill.subsp. *vulgare* var. *azoricum* (Mill.) Thell.) is one of the typical crops of the Mediterranean basin. Italy is the greater world-wide and European producer of *grumuli* based on 23700 ha and a production of about 590000 t (ISTAT, 2006).

Fennel is an open pollinated species belongs to the *Apiaceae* family and originated in the Mediterranean region, where it is possible to observe a high genetic variability. In the last few years the interest for a possible industrial use of fennel is growing. Nevertheless, this utilization would allow a diversification of the offer and the introduction of new products. Recently, fennel has become attractive for main international seed companies, which have improved research breeding programs. Moreover, changes of vegetable crops management and in the seed laws may have serious consequences on the maintenance of many local selections.

Considering the outcrossing rate and consequently the high genetic variability of this species more attention should be placed on the characterization of germplasm. The methods based on morphologic features commonly used not always allow the most accurate information due to genotypes-environment interaction; on the contrary it is well reported that genetic methods overcome this problems. Since not many genetic information are available in literature for fennel crop, microsatellites, frequently utilized for this purpose in many plant species, were adopted as molecular technique for fennel genetic characterization.

The aim of this study is the collection and characterization of several Sicilian fennel landraces, by using both morphological and genetic data. Eight fennel genotypes obtained from University of Catania (6 accessions) and from seeds bank of the University of Gatersleben (2 accessions), and one landrace named *finocchio riccio* were analysed. The genetic diversity is estimated and a matrix of presence/absence of DNA fragments is used for the comparison of the accessions in order to obtain the coefficients of genetic similarity. The coefficients are utilized for the UPGMA (Unweighted Pair Group Method Averages) analysis useful for obtaining a dendrogram among accessions.

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Poster Abstract – A.24

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF DIFFERENT ACCESSIONS OF *ORIGANUM VULGARE* L.

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Oregano, essential oils, M13-PCR

Oregano (*Origanum vulgare* L.) belongs to *Lamiaceae* family and it's an aromatic and medicinal plant widely used both as food source and phytotherapy. Moreover, it can be potentially used as biocide, because its essential oil is toxic against phytopathogenic bacteria, fungi and weeds. Essential oils are synthesized in glandular structures abundant on the leaf surface. The composition of essential oils is subjected to variations of the active principles, partially due to the use of heterogeneous populations and the environmental conditions.

The aim of this work is the comparison between oregano plants collected from different geographical areas and the effects of the environment. The plants were collected in the year 2006 in different areas of Campania region near Sicignano degli Alburni, Acerno, Ricigliano and Solofra and were cultivated in the Sele Valley (Salerno district). In the year 2007 the agronomic relieves at the harvest were carried out both on the plants in the origin areas and in the Sele Valley.

The results showed differences among the accessions and locations for bio-morphological characters, stomatic leaf density, biomass and essential oils yields. Flowering dates of the plants cultivated in the Sele Valley were earlier with respect to those ones grown in the places of origin. "Acerno" accession showed the highest height of the plants and weight of biomass with respect to the other accessions. The percentage of essential oil of "Sicignano" was higher than the other accessions both *in situ* and in the Sele Valley; conversely, "Acerno" accession showed the lowest percentage of essential oils in the two location. Anyway the essential oil percentage was higher in the original location for all the collected accessions.

M13 molecular characterization on the bulk of the four oregano accessions, was carried out. A very similar patterns among the accessions was observed, with only few differential bands. Further investigation on DNA polymorphism are in progress on single plants.

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Poster Abstract – A.25

SELECTION OF SPINELESS SAFFLOWER

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safflower, domestication, spineless

Safflower (*Carthamus tinctorius* L.) is an oilseed crop original from the semi-arid areas of the Near East and domesticated since long time for the production of an edible oil of good quality and the use of flowers as dyes for food and textile materials. The crop is characterized by the presence of very acute spines (Fig. 1) in the leaves and in the inflorescences, making the handling of the crop rather difficult.

45 accessions, obtained by one of us (A.B.) from Iran, India and USA, were grown in bulk and single completely spineless plants were selected. In the second generation these plants were segregates with and without spines and then only 50 progenies have been selected for the complete absence of spines. Besides this domestication trait, a large variability was maintained for many other morpho-physiological characters of the plants, such as plant height, branching, size of inflorescences, color of flowers, size, shape, color of seeds. Within this genetic pool further selection will be performed for oil content and fatty acids composition, in order to provide new cvs of this oil crop for fall seeding in marginal and semi-arid areas of southern and central Italy for both human alimentation and energy production.

Figure 1. Dried sample of *Carthamus tinctorius* L. showing the presence of spines on the top of the inflorescence.

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Poster Abstract – A.26

GERMPLASM CHARACTERIZATION AND FOOD TRACEABILITY OF *VACCINIUM* USING MOLECULAR MARKERS

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Vaccinium, microsatellites, characterization, Real Time PCR, traceability

Blueberry is currently cultivated in Italy on a relatively small scale, it is highly adapted to the Alpine regions and in Trentino (Northern Italy) commercial orchards the most important species are *V. corymbosum*, *V. angustifolium* and in a low portion *V. ashei*. The high concentrations of antioxidants and other beneficial health compounds in blueberries has increased the demand for this crop among health-conscious consumers and may further increase in the near future.

In commercial orchards, mismatched of blueberry accessions are very common, and quality techniques of fingerprinting are useful in order to guarantee the provenience given by nurseries and, at last, the quality standards of the production and yield.

To discriminate cultivars, using molecular markers, DNA was extracted using a commercial kit and amplified with a microsatellites primer set (30 loci) developed on EST (Expressed Sequence Tag) and genomic libraries. Standard microsatellite data analysis was carried out in order to classify all accessions and to have a reference for growers which can be used to prevent them from fraud.

To protect not only the growers but also the final consumers we extended the impact of our study even to food products which contains blueberry. A protocol developed on difficult matrices rich in polysaccharides, was used to extract DNA from food in order to detect the blueberry presence. The quality and quantity of specific DNA extracted and the discrimination from other genera was achieved using traditional PCR and Real Time PCR approach based on the application of microsatellites and other specific molecular markers.

Future work will be focused on the integration of molecular and pomological data in order to detect any association between loci and traits of interest which can be useful for MAS and breeding.

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Poster Abstract – A.27

MICROSATELLITE BASED POPULATION STRUCTURE OF ITALIAN MERINOS DERIVED BREEDS: A BAYESIAN APPROACH

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genetic diversity, microsatellites, sheep, Merinos derived breeds, population structure

In this study, both the genetic relationships and the population structure among Italian derived breeds (Gentile di Puglia, Sopravissana, and Merinizzata Italiana) and Spanish Merino using the Palmera sheep as outgroup breed, were investigated. Original Merino rams were first imported in Italy from the Aragon province as early as 1435 (rein of Alfonso D'Aragona) and continued from Rambouillet farm (France) until 1860. For this study, blood and hair samples were collected from 204 unrelated individuals belonging to 11 flocks and representing three derived Merinos breeds from southern-central Italy: Sopravissana (44), Gentile di Puglia (30) and Merinizzata Italiana (30). Spanish Merino (50) and Palmera Sheep (50) samples were also employed as reference and out-group breeds. All individuals were investigated for the genetic variation at 28 microsatellite loci (BM8125, BM1818, BM1824, CSRD247, CSSM66, ILSTS11, INRA6, MAF65, SPS115, TGLA122, BM6506, ETH225, ETH10, INRA35, INRA63, TGLA126, TGLA53, BM6526, OarCP20, OarCP34, OarFCB304, RM006, D5S2, HSC, MAF209, McM527, OarFCB11, OarFCB48) suggested by international organizations as the best for population genetic studies. Microsatellite markers were amplified with standard PCR reactions using seven multiplexes.

SSR data were used for cluster analysis according the MCMC algorithm to infer population structure and assign individuals to populations. A significant genetic relationship between Italian derived breeds and Spanish Merino was observed. The results show that the Italian derived Merinos breeds still contain a large genetic diversity and a clear population structure.

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Poster Abstract – A.28

COPY NUMBER AND TRANSCRIPTION LEVEL IN TRANSFORMANTS OF A WILD POTATO SPECIES (*SOLANUM CARDIOPHYLLUM)*

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copy number, transcription level, wild potato, Solanum cardiophyllum

In order to assay the effect of regeneration events on both transgene copy number and expression level, three different genotypes of *S. cardiophyllum* were used in genetic transformation experiments. In particular we used a wild type clone (CPH1C 2n=2x=24) and two regenerated derivatives, N11B (2n=2x=24) and C6B (2n=4x=48). *S. tuberosum cv.* Désirée (2n=4x=48) was used as control. *Agrobacterium*-mediated transformation was done according to either Millam (*In* : "Transgenic Crops of the World – Essential Protocols" pp 257-270, Ed. I. Curtis, Kluwer Academic) 2004) or Karp *et al.* (Plant Cell Tiss Organ Cult 3:363-373 1984) procedure by using a *p35S::GUS-INT* vector harboring the transgene *uidA* under a double 35S promoter. DNA and RNA were extracted from transformed plants by DNeasy Mini kit and RNeasy Mini kit, respectively. *UidA* copy number and mRNA relative abundance was assayed by Real Time qPCR.

Transgene copy number stably integrated in transformant genomes varied significantly between genotypes. On average, *S. cardiophyllum* transformants integrated more copies of *uidA* transgene than *S. tuberosum* (respectively 5.5 copies *vs.* 1.7 copies). The origin of explants affected the transgene copy number (Fig. 1). In fact, transfromants coming from the wild type CPH1C showed a significantly higher transgene copy number compared to transformats coming from N11B and C6B. Also, significant differences were observed in copy number between transformants coming from diploid and tetraploid derivatives, suggesting the transgene integration being affected by the ploidy level (Fig. 1). Analysis of larger samples of transformants will confirm this hypothesis.

UidA transcript showed significant variations between species. Again, *S. cardiophyllum* transformants showed higher level of the *uidA* transcript compared to *S. tuberosum* ones. In fact, *S. tuberosum* transformants performed an average relative quatification of 30.6 compared to *S. cardiophyllum* transformants with a relative abundance of the *uidA* transcript of 147.7. Within *S. cardiophyllum* transfromants, a strong effect of the origin of explants was observed on the *uidA* transcript abundance. Transformants coming from the wild type CPH1C showed a significantly higher transcript RQ when compared to transformants coming from N11B and C6B. Finally, transcript level was higher in regenerants deriving from 4X C6B than in those produced from 2X N11B. This work suggests that regeneration events may compromise the transgene integration through *Agrobacterium*-mediated transformation and limit the transcription of the transgene in further transformants. Our results also suggest that the ploidy level of explants may affect both transgene copy number and transcription level. Additional studies will investigate molecular

mechanisms elicited by somaclonal variations and affecting transgene expression in transformants coming from regenerated tissues.

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Poster Abstract – A.29

CHARACTERIZATION OF THE TOMATO LANDRACE "A PERA ABRUZZESE"

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genetic diversity, germplasm, landraces, Solanum lycopersicum

Aim of this study was to characterize 25 tomato accessions belonging to the landrace referred to as "A pera abruzzese", collected from various local farmers located in the Abruzzo region in central Italy. The accessions were grown in open field during the summer season of 2007 at the CRA-Agricultural Research Council in Monsampolo del Tronto (AP) using standard cropping practices. Eleven morphological-agronomic traits were recorded describing plant, flower and fruit morphology and productivity. At the molecular level, the accessions were analyzed at 18 selected Simple Sequence Repeat (SSR) marker loci. For the SSR analysis, seven control genotypes were added to the collection, representing both morphologically similar landraces (Canestrino di Lucca, Cuor di bue di Albenga) and very differentiated genotypes (San Marzano, Edkawi, Marmande, Spagnoletta).

The morpho-physiological analysis indicated that a considerable variability existed among the 25 accessions; in particular, variation was found for fruit characteristics, such as fruit shape (ranging from pear-shaped to globe-shaped, or even slightly flattened), size (ranging from less than 200 to more than 400 g) and ribbing (ranging from smooth to very ribbed).

Molecularly, the accessions were polymorphic at eight loci (where they yielded 29 alleles); the dendrogram based on genetic distances separated the "A pera" accessions from all the controls (including the Canestrino type), but not from the accession "Cuor di bue di Albenga". The adoption of a Bayesian grouping approach indicated that the collection was structured into three sub-populations, that only partially corresponded to distance-based clusters.

Taken together, the results suggest that the landrace "A pera abruzzese" represents a heterogeneous and dynamic population that includes a considerable variation for vegetative and reproductive traits. A thorough characterization, using both morphological and molecular descriptors, will help in defining the salient traits of the landrace, ascertain its distinctiveness from similar traditional varieties and put the basis for its inscription to the Register of Conservation Varieties.

This research was carried out with the contribution of the Abruzzo Region, project "Colture Orticole".

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Poster Abstract – A.30

MOLECULAR MARKER ASSISTED SELECTION TO INTRODUCE DISEASE RESISTANCE GENES IN TRADITIONAL TOMATO VARIETIES

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tomato, MAS selection, local germoplasm, disease resistance gene

Several traditional tomato ecotypes are cultivated on a small scale and to be better prized for their intense flavour. However, they lack disease resistance traits. Molecular marker are being widely used as a principal tool to introgress disease resistance genes in many crops. Marker-assisted selection programs (MAS) has proved to accelerate breeding program and to select in more effective way monogenic traits.

To this end, five tomato genotypes carrying various monogenic resistance genes were crossed with local adapted germoplasm (S. Marzano, Sorrento, Vesuviano and Parmitanella). The genotype are: Momor for the resistance to *Phytophthora infestant*, Stevens for the resistance to TSWV, Motelle for the resistance to *Fusarium oxysporum f. sp. lycopersici, Meloidogyne spp.*, and *Verticillium dahliae,* Pyrella for the resistance to *Pyrenochaeta lycopersici*, and Ontario for the resistance to *Pseudomonas syringae*. Various backcross schemes have been carried out starting from different F1 hybrids. At each backcross generation, the screening of resistant genotype was performed through molecular marker linked to the resistance genes. Up to date, BC3 generation has been reached for some cross combinations. Markers CAPS will be used for selecting the resistant genotypes. In the future we are going to combine different genotypes to obtain hybrids with multiple resistances.

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Poster Abstract – A.31

MARKER-ASSISTED SELECTION OF THE TYLCD RESISTANCE GENES *Ty-1* AND *Ty-2* IN TOMATO

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tomato, virus, Ty-1, Ty-2, marker-assisted selection

Tomato yellow leaf curl disease (TYLCD) is a devastating viral disease worldwide. Two species have been associated with epidemics in Italy: *Tomato yellow leaf curl virus* (TYLCV) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV). Genetic resistance is the most economic and sustainable way to control this disease. Several sources of resistance have been discovered in wild tomato species. However, classical selection has been proven to be slow and difficult. This study is focused on the development of traditional Italian varieties of tomato resistant to TYLCD. In order to investigate the effectiveness of two of such resistance loci, we screened lines LA3473 and H24, carrying respectively *Ty-1* and *Ty-2* genes, against TYLCD isolates collected in tomato production regions in the south of Italy. *Ty-1* gene has shown to provide tolerance to TYLCSV isolate whereas *Ty-2* has proven to be fully effective against TYLCV isolate. Two CAPS markers linked to each gene, TG178 and TG436 for *Ty-1*, TG105A and C2_At5g25760 for *Ty-2*, were screened for their utility in marker-assisted breeding programs. F2 populations from crosses between resistant and susceptible lines were marker-analysed and selected F3 progenies were phenotyped for their resistance.

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Poster Abstract – A.32

DEVELOPMENT OF NUTRITIONAL AND AGRONOMIC INDEX AS TOOL TO SELECT NEW TOMATO HYBRIDS

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hybrids, yield, quality, resistance genes, tomato

Fruits and vegetables play a significant role in human nutrition. Among vegetables, tomato is the most important both for its large consumption and for its richness in health-related food components. This vegetable is an important component of traditional Mediterranean diet, but also of other diets. There is evidence that regular tomato consumption decreases the incidence of chronic degenerative diseases, such as certain types of cancer and cardiovascular diseases. Epidemiological findings confirm that the observed health effects are due to the presence of different antioxidant molecules such as carotenoids, particularly lycopene, ascorbic acid, vitamin E and phenol compounds, particularly flavonoids. For long time, fresh market tomato breeders have improved yield, resistance to diseases, and fruit aspect but have lacked clear targets for improving fruit quality.

In the last few years, one of the main objectives in tomato breeding programmes was selecting genotypes with high nutritional value. In sight of this, seventeen tomato lines were analyzed for nutritional quality proprieties and agronomic traits. They comprised seven parentals and ten derived tomato hybrids. In particular, from the nutritional point of view, 8 components contributing to the healthy quality of tomato (i.e., lycopene, β-carotene, other carotenoids, flavonoids, phenolic acids, vitamins C and E, dry residue) were assessed. From the agronomic point view, performance as total yield and number of commercial fruits, were analyzed. Selection of hybrids was based on the development of two indices: a tomato nutritional index, denominated IQUAN, and a agronomic index that considered also the of presence resistance genes in analyzed lines. Combining the two index, two hybrids (MR 48 and MR 47) merit high interest as tomato genotypes with considerable amounts of vital antioxidants and an acceptable commercial production.

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Poster Abstract – A.33

A COMMON ANCHORED MAP BASED ON A FRAMEWORK OF COSII MARKERS FOR POTATO AND A SET OF TOMATO "EXOTIC LIBRARIES"

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introgression lines, wild species, COS II markers, genetic maps

Genome mapping results and their recent applications have demonstrated that the genetic diversity stored in germplasm banks can be utilized much more efficiently than before. Therefore, a major objective in modern breeding is to return to natural biodiversity resources, in the form of wild species or old varieties, and employ some of the diversity that was lost during domestication and breeding for the improvement of the agricultural performance of modern varieties. In this respect, "exotic libraries", which consist of sets of segmental introgression lines (ILs), each carrying a single marker-defined genomic region that derives from a donor wild species in an otherwise uniform elite genetic background, have proven to be very efficient tools for plant breeders. Comparative mapping has also shown extensive genome colinearity among plant species of the same family. Therefore, in order to enhance the rate of progress of breeding based on wild species resources, and to facilitate comparisons between function maps of tomato and potato, "exotic libraries" of tomato from a diverse selection of accessions (including *S. pennelii* LA0716, *S. habrochaites* LA1777, *S. chmielewskii* LA1840, *S. neorickii* LA2133) and a diploid mapping population of potato are being anchored using a common set of Conserved Ortholog Set II (COSII) markers (http://www.sgn.cornell.edu/markers/cosii_markers.pl). This work is being conducted within the framework of the EU-SOL project (http://www.eu-sol.net/). The construction of a common PCR-based marker framework, which links the tomato and potato maps, the genetic infrastructure of tomato "exotic libraries", along with the numerous qualitative and quantitative traits mapped in both species, should facilitate quantitative trait locus (QTL) identification, additional mapping, cloning of the underlying genes and the use of the novel variation in marker-assisted breeding.

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Poster Abstract – A.34

PHENOTYPIC AND QUALITATIVE EVALUATION OF FIELD BEANS *(VICIA FABA* SPP.) POPULATIONS

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Vicia faba spp., field beans, phenotypic and qualitative evaluation

Vicia faba spp., originated in the Mediterranean-West Asia region during the Neolithic period and now cultivated in many temperate regions, is one of the oldest legume crops mainly grown for human and animal dietary needs. This crop like other grain legumes, contributes to sustainable agriculture by fixing atmospheric nitrogen. Faba bean has been divided by seed size into three subspecies. The broad bean (*V. faba var. major* Harz) is mostly grown as a grain vegetable because of its large seed size, while the horse bean (*V. faba var. equine* Pers.) and tick bean (*V. faba var. minor* Beck) are grown primarily for animal feed or as a green manure crop; in Europe, these two latter species are referred to as field beans. In the Mediterranean area these species are very important. Disappointingly cultivation of these crops has decreased in the last decades, although in recent years their importance is increasing due to difficulties in sourcing GM-free soya beans and also for their use in organic farming.

Faba beans breeding has proceeded very slowly and with only few interesting results. More than 90 cultivars of *V. faba spp.*, half of which are Dutch, 18 British and 15 Italian, are registered in the European Community Catalogue. However most of the Italian varieties were registered before 1990. Improvement in seed yield and yield stability are the primary objectives of most faba bean breeding programmes. However other objectives such as resistances to the main biotic and abiotic stresses, obtaining genotypes that are free of certain anti-nutritional substances are also important. In the present work, data on the main morpho-agronomic traits and chemical and nutritive characteristics are recorded and determined on landrace accessions of field beans belonging to a collection established in the Umbria Region. The results obtained in the material analyzed enabled us to identify interesting genotypes for further breeding programs.

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Poster Abstract – A.35

ORIGINS AND DOMESTICATION OF *PHASEOLUS VULGARIS*, AS REVEALED BY CHLOROPLAST AND NUCLEAR MOLECULAR MARKERS

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Phaseolus vulgaris, domestication, plastidial and nuclear diversity

The common bean (*Phaseolus vulgaris* L.) is the most important legume for direct human consumption, and in some countries it is the primary source of protein in the human diet. From a population genetics perspective, the major subdivisions of wild common bean progenitors are known, and the domesticated gene pools have been defined. Two major domestication events, one in Mesoamerica and the other in the southern Andes, appear to have resulted in the Mesoamerican and Andean gene pools that mirror the geographic distribution of the wild progenitors. In the present study, we have analyzed 190 genotypes of *P. vulgaris*, which are representative of all of the different gene pools and forms (wild and domesticated). All of the individuals were analyzed using 17 chloroplast microsatellites (cpSSRs); due to their relatively high levels of polymorphism and their generally uniparental inheritance, cpSSRs represent a useful tool for the study of genetic variation and evolution in plants. A subset of 131 genotypes was also analysed by nuclear markers, such as AFLP (300 polymorphic markers), distributed along the whole genome, and two STS designed on the genomic sequence of *Pv*-SHATTERPROOF1, which is similar to SHATTERPROOF1 of *Arabidopsis thaliana*. The results are discussed in relation to the origins and domestication of *Phaseolus vulgaris*.

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Poster Abstract – A.36

BIODIVERSITY STUDIES IN *PHASEOLUS* SPP. BY DNA BARCODING

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common bean, cpDNA, ITS, genetic diversity, SNPs

DNA barcoding is a technique for identifying species by obtaining a short DNA sequence from a known gene and comparing it with databases of orthologous sequences from species of established identity. Our study deals with the use of DNA barcoding as a new tool to assess genetic relationships among *Phaseolus* species and genetic distinctiveness of *P. vulgaris* varieties. While in a range of animals, the mitochondrial genes, such as the COI, have been proved to be suitable for DNA barcoding, in other organisms they are not useful. Land plants, especially angiosperms, seem to be problematic for DNA barcoding since most mitochondrial DNA regions have exceedingly low levels of variation to distinguish between taxa. The mitochondrial genome in plants undergoes significant rearrangement and horizontal transfer of genes, both at intra and interspecific levels. Standard genic and intergenic regions from the chloroplastic genome can offer for DNA barcoding in plants what the mitochondrial genome does for animals: it is an uniparentally inherited, non recombining and structurally stable genome.

A total of 54 accessions of *Phaseolus vulgaris* were arbitrarily selected as representative of gene pools on the basis of passport information and previous molecular investigation data, along with a few *P. coccineus*, *P. lunatus* and *Vigna unguiculata* accessions adopted as reference standards and out-types. In particular, 24 Italian pure lines, 18 Mesoamerican landraces and 12 Andean landraces of *P. vulgaris* were characterized by amplifying and sequencing four plastid genic regions (rbcL and trnL) and intergenic spacers (atpB-rbcL and psbA-trnH), along with the nuclear internal transcribed spacers (ITS1 and ITS2). The experimental strategy included the following steps: i) retrieving nucleotide sequences of the selected DNA regions from the NCBI databases in the *Fabaceae* family; ii) performing serial local multiple sequence alignments; iii) designing of specific primer pairs in highly conserved short stretches (300-500 bp) flanking the most variable regions; iv) characterization by amplifying and sequencing of the distinct cpDNA regions along with the ITS1-ITS2 for rDNA regions; v) editing and alignment of sequences by Sequencer software; vi) clustering of sequences by UPGMA and NJ methods supported by bootstrapping analysis using PAUP software for phylogenetic analysis.

On the whole, all designed primers proved to be highly specific for the amplification of target DNA sequences. The occurrence of SNPs in either *Phaseolus* spp. or *P. vulgaris* was much lower in plastid DNA sequences than nuclear ITS regions (overall, 36 vs. 72 and 5 vs. 10, respectively). Most importantly, a total of 17 different haplotypes were identified for the 54 common bean accessions. It is worth mentioning that most domesticated Andean varieties were clustered apart from Mesoamerican varieties, whereas wild Andean and Mesoamerican accessions were grouped into two tightly related subclusters. In conclusion, the DNA barcoding confirmed to be a very

powerful technique for phylogenetic purposes in plant species, but revealed to be poorly informative for the genetic traceability of single plant varieties. As a matter of fact DNA barcoding provides an accurate method for the genetic identification of bean species by using a standardized genic or integenic region as molecular tag. The research is in progress with the main goal of discovering additional SNPs and reconstructing haplotypes to be exploited for the identification of varietal groups.

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Poster Abstract – A.37

ORIGIN AND STRUCTURE OF THE EUROPEAN COMMON BEAN (*PHASEOLUS VULGARIS* L.) LANDRACES

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c*hloroplast microsatellites, genetic diversity, gene pool, bottleneck*

Domestication of *Phaseolus vulgaris* L. occurred independently in Mesoamerica and the Andes, giving rise to two highly differentiated gene pools. The pathways of dissemination of beans into Europe were very complex, with several introductions from the New World combined with direct exchanges between European and other Mediterranean countries. In the present study, we have used seven chloroplast microsatellite markers (cpSSRs), and two unlinked nuclear loci: phaseolin and *Pv-SHATTERPROOF1*. The molecular data were used to assess the genetic structure and the level of diversity of a large collection of European landraces of *P. vulgaris* (307) in comparison with 94 American genotypes representing the Andean and Mesoamerican gene pools. We then compared the diversity of common bean landraces from Europe (as numbers of alleles, haplotypes, gene diversity and genetic differentiation) with that from the American centres of origin. Our results show that most of the European common bean landraces are of Andean origin and that the bottleneck due to the introduction into the Old World was not as strong as has been previously suggested. Finally our data indicate that in Europe, a significant portion of the bean germplasm has derived from hybridization between the Andean and Mesoamerican gene pools.

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Poster Abstract – A.38

CHARACTERIZATION OF A BEAN LANDRACE FROM SICILY: THE 'FAGIOLO BADDA DI POLIZZI'

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Badda bean, genetic diversity, germplasm, landraces, Phaseolus

Inside the project "Piano per la Produzione di Proteine Vegetali in Sicilia" investigations were carried out to characterize a bean population cultivated in the Parco delle Madonie area, the 'Fagiolo Badda di Polizzi'. The producers' interest for this landrace and the appreciation of the consumers could justify the start up of a valorization program through a product certification.

Researches on the morpho-physiological aspects and on the nutritional profile were made in trials conducted between 2005 and 2007. All the Badda bean accessions showed an indeterminate plant growth habit, white flowers and a very delayed flowering time. In particular, a certain variability was seen and described for the size and shape of pods and seeds. Two types of Badda are cultivated, differentiated by the secondary seed colour: the "white Badda" and the "black Badda". Both have ivory has a primary seed coat colour, but the "white Badda" has a brownish spot on the hilum, whereas the "black Badda" shows a black spot. In addition, the secondary colour of the seed coat of the "black Badda" showed two different pigmentations: violet and black, that suggested a genetic differentiation into different sub-populations. Finally, the "black Badda" resulted less susceptible to viral infections than the "white" one.

At the molecular level, three accessions of Badda bean (two 'white' and one 'black') have been compared with control varieties, including one accessions of 'Fagiolo del Purgatorio' from Gradoli (VT), seven landraces of the Borlotto type collected in the Marche region and the cultivars Bat, Jalo, Clio and Big Borlotto. The analyses were carried out using 12 Inter Simple Sequence Repeats (ISSR) primers yielding a total of 140 bands. Although no specific band for the Badda landrace were detected, two amplicons were found only in the accessions of Badda and in Monachello, a bicol-seeded type from the Marche region, morphologically similar to the "black Badda". The dendrogram obtained from the genetic distances based on ISSRs indicated that the Badda type belongs to the Andean gene pool and that it is distinguishable from the tested controls being grouped into a separate cluster. Within the Badda type, the 'white' accessions were not separated from the 'black' one.

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Poster Abstract – A.39

RECOVERY AND USE OF LENTIL LANDRACE "VILLALBA"

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ecotype, lens culinaris, landrace, morphophysiological characteristics, sanitary characteristics

Lentil is one of the most widespread grain legumes in the Mediterranean area. In Italy, the cultivation of this legume has ancient origins and over the centuries, has been domesticated and adapted into two distinct morfotypes: one with large seed and another with small seed. In the last fifty years the areas grown with lentil, decreased from 25,000 ha to the current 1,800 ha. In this context, only a few productions localized in typical well-defined areas have remained.

This work has been done to enhance a local population of lentil, cultivated in "Villalba" (CL). The study, conducted in the three years 2005-2007, has focused attention on morfophysiological characterization and monitoring of plant health. The results show a good degree of homogeneity and stability for the main morfophysiological characteristics. In the different years and in the different fields under investigation, no phytosanitary issues of importance have been found. The realization of this description lays the groundwork for unmistakably distinguish the local population of "lentil Villalba" and safeguard its geographical area of production, also considering the absence of phytosanitary problems.

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Poster Abstract – A.40

USE OF BIODIVERSITY FOR THE DEVELOPMENT OF NEW LENTIL WINTER LINES WITH QUALITY TRAITS

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lentil, genetic diversity, quality, tolerance to abiotic stresses

In Italy, lentil (*Lens culinary* Med.) is considered a minor legume and the grain yield is low and aleatory. Therefore, due to different causes such as the change of alimentary customs, this crop suffered in the years a progressive and drastic decrease of interest. Apart from the recent release of three new varieties, heterogeneous local populations and foreign varieties are still today cultivated.

According to the exigence of improving the cultivated populations and developing varieties with desirable traits, our research has attempted to study the existing genetic diversity and to identify winter lines in lentil germplasm collections.

Selections within the new germplasm have been carried out from a morpho-physiological point of view. Lines previously selected in Viterbo have been assessed for tolerance both to high and low temperatures in Battipaglia (Salerno) and Montefalcone di Val Fortore (Benevento), respectively. The genotypes selected in the three different environments have been agronomically evaluated. The best lines were characterized by seed quality analysis. Germplasm surviving to the evaluations could be suitable as potential crossing material for both agronomical and qualitative traits. Hence, it could be useful either as donor of these traits or as lines for release of new commercial cultivars.

Results provided a picture of lentil biodiversity related to various characters (biological cycle, plant height, erect *habitus*, lodging, tolerance to unfavourable environmental conditions, size, colour and weight of the seeds, etc.) among the germplasm examinated and showed that there were marked differences in quantitative seed production and quality traits.

Microsperma and *macrosperma* lines, characterized by early, erect *habitus* for mechanical harvesting and high yielding plants, in some cases tolerant to high or low temperatures, have been selected and some of them showed also a good seed quality.

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Poster Abstract – A.41

MOLECULAR FINGERPRINTING OF ASPARAGUS ACCESSIONS *VIA* MICROSATELLITES AND ISOENZYME-DERIVED POLIMORPHIC SEQUENCES

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molecular markers, Asparagus officinalis, hybrids, doubled haploids

The overall goal of asparagus breeding in Italy is the development of all-male hybrids (HF1) from the combination of doubled haploid clones (Falavigna et al, 2002). The characterization of six HF1 commercial hybrids (Eros, Marte, Ercole, Giove, Italo, Zeno), their parents and the outgroup accession "Montina", belonging to *Asparagus maritimus* Miller, was performed by means of Simple Sequence Repeats (SSR) generated from a public expressed sequence tag (EST) collection (Caruso et al, 2007). All genotypes were also phenotypized and analyzed by means of isozyme-derived polimorphic sequences following the protocol developed by Wang and coworkers (2007) for the generation of new molecular markers. The isozymes were chosen on the bases of results obtained by Gonzales-Castañon (1999) and Falavigna and coworkers (2008). The sequences of the most polymorphic isozymes were retrieved from public databases for the analysis of conserved regions.

The results show the opportunity to use SSR and isozyme-derived PCR-based markers together with phenotypic descriptors for the univocal identification of asparagus hybrids. Furthermore, the development of PCR-based markers from isozyme gene families gives the access to a large amount of "old" data for the development of new molecular markers.

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Poster Abstract – A.42

THE FIRST FOUR CLONES SELECTED FROM THE TRADITIONAL ARTICHOKE ROMANESCO POPULATIONS

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artichoke, Cynares, Cynara, variety, descriptor

The 'Romanesco' variety, with its spherical or sub-spherical, non-spiny green-violet heads, accounts for about 9% of Italy's production (ISTAT 2005). It includes two important populations: Castellamare and Campagnano. The former is an early-maturing type while the latter is a late-maturing type. Recently, the C3 clone, which matures earlier than the Castellamare, has replaced much of the 'Romanesco' landrace material. This has led to a significant erosion of local genetic resources and a loss of diversity. In order to conserve and safeguard artichoke germplasm of Castellammare and Campagnano types, ENEA and Tuscia University collected germplasm from farmers' fields which is currently conserved both *in vitro* and in field gene banks. The germplasm is being evaluated for morphological, molecular and quality traits. The conservation and evaluation activities are now continuing within the framework of the CYNARES project, receiving financial support from the European Commission, DG for Agriculture and Rural Development, under Council Regulation (EC) No 870/2004. The project brings together skilled partners from leading European countries in the field of *Cynara* research for several propositions, including germplasm rationalization and the increase of new accessions.

To assure germplasm variability and to protect some landrace typologies, 4 'Romanesco' clones will be presented for release at the National Variety Register in collaboration with ARSIAL (Agenzia Regionale per lo Sviluppo e l'Innovazione dell'Agricoltura nel Lazio).

The clones, which will be fully described in the poster, are characterized by differences: maturity dates, ranging from beginning to end of March; size of head (diameter from 8 to 10 cm); production weight (from 1.7 to 2.3 kg); receptacle thickness (from 0.5 to 0.9 cm); and molecular patterns. The 4 clones belong to Campagnano, Grato 1, C3, and Castellamare typologies: Campagnano is characterized by late maturity (end of March), tall and large plants, absence of spines, medium-sized heads and a short period of production (within about 23 days); C3and Castellamare are characterized by early maturity (beginning of March), short plants, good production in terms of total weight but differently distributed between primary and secondary heads, thick receptacles, uniformity in the shape of the tip, the head and the presence of mucron; Grato 1 is characterized by big and heavy heads, mid maturity (mid March) and medium-large plant size, dark colour, good production in terms of total weight within 28 days of production.

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Poster Abstract – A.43

CONSTRUCTION OF A NEW GENETIC LINKAGE MAP OF *CYNARA CARDUNCULUS* L.

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globe artichoke, cardoon, linkage analysis, genetic map, pseudo-testcross

Cynara cardunculus (*Compositae*, 2n=2x=34) is a perennial allogamous species, native to the Mediterranean Basin, with an estimated genome size of 1078Mbp. It includes globe artichoke (var. *scolymus* L.), cultivated cardoon (var. *altilis* DC.) and their progenitor wild cardoon [var. *sylvestris* (Lamk) Fiori]. *C. cardunculus* improvement through breeding has been rather limited and, unlike other crop species belonging to the same botanical family (such as sunflower, lettuce and chicory), its genome organization remains largely unexplored. To move towards a modern breeding it is compulsory to generate genetic maps for identifying the genetic bases of key resistance and agronomic traits. Due to the high level of heterozigosity of the species, and the marked inbreeding depression following selfing, the most suitable strategy for linkage analyses is the two-way pseudo-testcross.

We produced three F_1 progenies obtained by crossing a globe artichoke clone of 'Romanesco C3', as female parent, with three pollen sources: a genotype of globe artichoke 'Spinoso di Palermo' (progeny A); one of cultivated cardoon (progeny B) and one of wild cardoon (progeny C). The first genetic maps were developed by genotyping progeny A and applying AFLP, M-AFLP, retrotransposon based SSAP as well as the first available set of SSR markers. They comprised 204 (female map) and 180 (male map) loci spread over 18 and 17 linkage groups respectively; furthermore, the presence of 78 markers in common to both maps allowed the alignments of 16 linkage groups. Recently a new set of microsatellites has been developed and 35 SSR loci were suitable to implement these maps, of which 19 shared between parents.

Here we report on the development of a new consensus map on progeny B, based on more than 700 AFLPs and 60 SSRs markers, the latter representing a set of robust and informative anchor points between the segregating populations. Another map, based on progeny C, is currently under construction and markers suitable for mapping in the three F_1 progenies will be used as point of reference to locate important genes to a particular LG.

C. cardunculus is easily vegetatively propagated, thus the mapping populations are immortalised, and are at present growing in contrasting environments to investigate genotype x environment interactions. The generated maps will open the way for QTL analyses and future application of a marker assisted selection breeding strategies.

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Poster Abstract – A.44

NUCLEAR AND CHLOROPLAST DNA VARIABILITY IN *ARUNDO* (ARUNDINOIDEAE)

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biomass, chloroplast, phylogenesis

In the Mediterranean environment several perennial grasses are the leading candidates to become energy crops because they produce lignocellulosic biomass that is ideal for fuel and because they also display a good adaptability to such environments. Among perennial grasses, *Arundo donax* L. (giant reed) grows spontaneously and abundantly in Lombardy, with rapid growth and high yield capacity. Moreover, giant reed has attracted attention due to other potentially benefits such as phytoremediation and landscape beautification. Giant reeds produces flowers but viable seeds have not been observed in most areas where it has been introduced. Agamic propagation by rhizomes is the principal way to plant this species and one of the most important problems to be solved is the high planting costs, due to the difficult mechanisation of the propagation practices by rhizomes. On the other hand, in other areas of world, giant reed has escaped cultivation and become a invasive weed of riparian habitats where it not only displaces native species but also modifies ecological and successional processes. In this study, we investigate genetic variation in *A. donax* and in putative and fertile parent species (*A mediterranea, A. plini and A. collina*) using ISSR primer and chloroplast spacers (Rps16-TrnK, Rpl32-TrnL, PsbA-TrnH). The real genetic diversity of giant reeds at a wider geographical scale is not known. The knowledge of the level of genetic variability is particularly important in breeding and/or biological control programs.

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Poster Abstract – A.45

POLYPLOIDIZATION: EFFECT ON THE TRANSCRIPTOME AND METHYLOME IN *MEDICAGO SATIVA*

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Medicago sativa, microarray, MSAP, polyploidy, Solanum tuberosum

The modifications that occur as a consequence of polyploidization in plants can influence economically important traits. These modifications may be caused by changes in gene expression and/or in DNA sequence. While the genetic and epigenetic effects of polyploidization have been studied in both model and cultivated disomic polyploids, polysomic polyploidy has not received much attention. The objective of our work is to gain insight into the effects of chromosome doubling on gene expression and DNA methylation in alfalfa, an important forage species with tetrasomic inheritance. We used two diploid (2x=16) plants of the subspecies *Medicago falcata* and *M. coerulea* that produce 2n eggs and 2n pollen, respectively. From their cross, diploid and tetraploid (4x=32) progenies from bilateral sexual polyploidization (BSP) were obtained. We have used three 2x and three 4x progeny plants to investigate polyploidization-induced changes by analyzing: 1) gene expression for a large part of the genome, by using microarrays of cDNA-derived sequences of *Medicago trucatula* and *Medicago sativa* (Affymetrix), and 2) genomic DNA methylation, by using the Methylated Site Amplified Polymorphism (MSAP) technique. Microarray analysis has evidenced about 200 genes differentially expressed between 2x and 4x progenies. Fifty of them appear to change expression as a consequence of polyploidization. A full Gene Ontology analysis of these genes was performed. MSAP analysis has been initiated using selective primers capable of a high-polymorphism detection, based on previous assays on alfalfa. Our preliminary results revealed some methylation changes in the BSP progenies with respect to their 2x parents. Dry matter yield and fertility of the parents and progenies were compared and discussed in the light of the molecular data obtained.

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Poster Abstract – A.46

HETEROZYGOSITY TREND ESTIMATED BY SSR MARKERS IN THE FIRST STEP OF ALFALFA FREE-HYBRIDS CONSTRUCTION

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alfalfa, selfing, free simple hybrids, SSR markers

In the construction process of 4-constituents alfalfa free-hybrids (Rotili et al., 1999), the first step is the crossing of partially inbred (S_2) parental plants, selected for dry matter production and other traits of interest during two cycles of selfing, to obtain Simple Hybrids (SH) S_2xS_2. These hybrids, once multiplied with selection till Syn3 generation, will constitute the parents ($2S_2Syn3$) of the 4-constituents free-hybrids.

Aims of the present work are to study the genetic diversity of the S_2 parents and to estimate the trend of heterozygosity recover following the crossing S_2xS_2 by means of SSR molecular markers; besides, to investigate the relationship between these parameters and the yielding performance of SH progenies.

The parental population was represented by S_1 progenies of plants from somatic hybridization *M. sativa* x *M. falcata* (Téoulé, 1983) crossed to non-inbred *M. sativa* of various origins. Fourteen S_2 mother plants (MP) belonging to 4 families were chosen by means of positive selection for dry matter (DM) yield and total stem height and divergent selection for average internode length during two selfing cycles. SH progenies were obtained by manual crossing without emasculation of the S_2 MPs and the corresponding synthetics (Syn2 generation) by manual polycross of the same plants. Four SHs (640 plants in total) and the corresponding synthetic (1600 plants) were studied for two years in miniplots 25 cm diameter 80 cm height at the density of 400 plants m^{-2} with not limiting irrigation. Within each SH progeny, the best performing individuals (mean + 1.5*s*) with the desired stem morphology were selected (5, 6, 3 and 3 respectively). The 17 SH individuals and the 14 S_2 MPs were analyzed by means of 67 SSR loci derived from both *M. sativa* and *M. truncatula* to estimate genetic similarity (DICE coefficient) and heterozygosity level.

The four Simple Hybrids differed significantly for total dry matter yield (11 cuts in two years): with reference to the production of the corresponding synthetic (Syn2), the best SH showed a gain of +30% and the weakest one a decrease of -28% . Final mortality was consistent with the productive data resulting 5% and 47.5% respectively for the highest and the weakest SH. The analysis of SSR data is currently being performed.

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Poster Abstract – A.47

SPATIAL GENETIC STRUCTURE OF *TAXUS BACCATA* L. IN THE WESTERN MEDITERRANEAN BASIN: PAST AND PRESENT LIMITS TO GENE MOVEMENT OVER A BROAD GEOGRAPHIC SCALE

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phylogeography, nuclear SSRs, genetic structure, conservation

The knowledge of biodiversity and the implementation of strategic plans for its sustainable use, particularly concerning threatened species and habitats, is one of the main National and International priorities for Research and Development. In this framework, the study of the genetic diversity, and the analysis of the historical, evolutionary and ecological factors and processes that determine its distribution, contributes to the design of sound management policies for its use and conservation.

Long-term management strategies for conservation of yew (*Taxus baccata* L.) populations require the assessment of the distribution of genetic resources at different spatial scales. Therefore, knowledge is needed about the spatial organization of its genetic variation, as well as about gene flow and their relationship with fragmentation and/or isolation in this species. Therefore, we have developed 7 specific microsatellite loci for *Taxus baccata* in order to investigate the genetic structure of this species at different spatial scales: wide-range scale, Mediterranean scale, local scale. Our main objectives are: (i) to know if there are particular areas or geographical regions with different levels of genetic variability and structure; (ii) to assess the role of historical processes in determining such patterns; and (iii) to understand the interaction between the demographic (ecologic) and genetic dynamics under different scenarios of landscape fragmentation.

In this communication we present the results obtained so far for the populations located in the western Mediterranean. We discuss the role of the complex paleogeographic and paleoclimatic history of this region in the current distribution of genetic diversity. Finally, it is expected that these results will help in designing appropriate management strategies for its conservation.

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Poster Abstract – A.48

GENETIC DIFFERENTIATION IN SCOTS PINE (*PINUS SYLVESTRIS* L.): A COMPARISON BETWEEN POPULATIONS FROM ITALY AND PART OF THE REMAINING EURASIAN NATURAL RANGE

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population genetics, Pinus sylvestris, genetic diversity, genetic differentiation, natural range

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

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Poster Abstract – A.49

CONSERVATION AND GENETIC DIVERSITY IN *JUNIPERUS SPP* OF "POLLINO NATIONAL PARK" (BASILICATA, SOUTHERN ITALY)

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Juniperus nana, Juniperus hemispherica, SSRs, genetic diversity, conservation

The shrub vegetation of high mountains of Pollino National Park, (Lucanian side) is characterised by small procumbent junipers (*Juniperus nana, J. hemispherica* - Cupressaceae) forming large carpets in upper level of impassable areas frequently together with other shrubs and Bosnian pine (*Pinus leucodermis* Antoine). They are drought and frost tolerant, although sensitive to fire. Since the Bronze Age to nowadays shrubberies suffered a slow decline and they have survived in refuge sites difficult to reach as rocks, plateaus, ridges of high mountains. In the last decades climatic changes could start a new regressive phase promoting the advance in upper level of mesophilic hardwood forests. Moreover Junipers of upper level are regarded as species critics in taxonomic fields. Both of the Junipers growing in the Pollino areas, for their rarity and vulnerability need to be preserved and propagated. For their longevity and tolerance of environmental stresses they are interesting for nursery purposes too.

Within Terranova del Pollino area (Potenza, Italy) three sites were identified at different altitudes, from 2051 m a.s.l. site in Sic IT9210245 area (Serra di Crispo, Grande Porta di Pollino and Pietra Castello) to 1640 m a.s.l site of 'Piano Jannace'. Ten female individuals from each site were sampled. Genetic diversity between and within sites was studied by means of five nuclear SSR loci. The SSRs were identified among those already developed in different species within the family *Cupressaceae*: 4 specific for *Juniperus communis* and 1 for *Cupressus sempervirens*. 4 out of five microsatellites were polymorphic and over all 37 alleles were identified from 3 to 13 alleles per SSR locus. Variation among sites was also studied.

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Poster Abstract – A.50

IN VITRO SCREENING FOR RESISTANCE TO APPLE PROLIFERATION IN ROOTSTOCK BREEDING MATERIALS

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Malus spp, breeding, apple proliferation, micrografting, quantitative real-time PCR

A breeding program was started six years ago in Trentino region within the Project Scopazzi del Melo – Apple Proliferation (SMAP) in order to obtain AP resistant apple rootstocks suitable to modern fruit growing. Twenty cross combinations used *Malus sieboldii* and its hybrids with *M. domestica* as donors of the resistance trait. Despite different degrees of apomixis and polyploidy, 3300 individual plants were obtained, even though not all were genuine hybrids. Seedlings were successfully analysed with molecular markers and flow cytometry so that mode of reproduction, genomic constitution and ploidy level were inferred. Sets of 5-6 locus specific microsatellite markers right for characterizing each progeny were applied. A screening system for AP resistance was developed, based on *in vitro* graft-inoculation with the causal agent '*Candidatus* Phytoplasma mali'. The phytoplasma concentration in inoculated shoots was determined at different times post-inoculation by quantitative real-time PCR. All infected resistant parents had lower phytoplasma concentration than the susceptible controls. They did not show AP-specific symptoms and their growth was not affected. The resistant behaviour of *M. sieboldii* and some of its hybrids (H0909, D2212) infected with two different strains (PM4 and PM6) was confirmed *in vitro.* Preliminary results show that resistance trait segregates in the progenies.

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Poster Abstract – A.51

ISOLATION OF SSR MARKERS TIGHTLY ASSOCIATED TO THE VM APPLE SCAB RESISTANCE GENE

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resistance gene, plant disease, V. inaequalis, microsatellite, apple

The hypersensitive reaction is a resistance mechanism in which after cellular contact between pathogen and the host, a plant reaction can be observed, usually leading to the fast death of the pathogen's underlying cells. Hypersensitivity-based resistance is well studied in many model systems: basically, the defence cascade, which often ends macroscopically with hypersensitivity and biochemically with the synthesis of phytoalexins, is activated through host recognition of the pathogen. In *Malus* this is the case of *Vm* resistance. Historically, Dayton and Williams (1970) used *Vm* to denote the resistance gene conditioning the pit-type HR reaction carried by *M. micromalus* 24538 and *M. atrosanguinea* 804. The interest around this gene derives not only from its evident, typical and fast hypersensitive response, but also from the fact that this is a monogenic resistance, relatively easy to be introgressed in commercial apples.

Two molecular markers—OPB12SCAR (Cheng *et al*., 1998) and SSR Hi07h02— tightly linked to *Vm* gene have already been reported (Patocchi *et al*., 2005). The SSR marker allowed the mapping of *Vm* on linkage group 17 in a region where also scab resistance QTL have been reported (Durel et al., 2004).

Considering all these knowledge, a program to create the conditions for a rapid isolation of the apple scab resistance gene *Vm* was started.

Large *Vm*-segregating populations have been created, phenotyped in greenhouse after pathogen inoculation and genotyped using molecular markers associated with the *Vm* resistance gene. A Florina BAC library from Vinatzer et al. (1998) has been pooled to perform an 'heterologous' chromosome walking aimed at identifying new markers tightly linked to the *Vm* gene. The BAC library screening with the Hi07h02 marker resulted in the identification of three BAC clones carrying the marker alleles of Florina. The BAC-ends of these clones were sequenced and both used to develop new SSRs tightly linked to the *Vm* gene and to perform a second chromosome walking step. Two new microsatellites showing an high level of polymorphism in different populations (Fiesta x Discovery, Golden Delicious x Murray, Galaxy x Murray) were developed and mapped in the distal part of the LG 17. One of these new SSR markers (Vm1SSR) showed no recombinants among 1260 tested seedlings and the other (Vm2SSR) showed only 6 recombinants. Genetic distances from *Vm* were 0 and 0.47 cM, respectively therefore the resistance gene has been located between Vm2SSR and the SSR Hi07h02 (this marker showed 7 recombinants).

A new BAC library from cv. Murray DNA (carrying the *Vm* resistance gene) was constructed and pooled in order to identify clones containing the genomic region containing the *Vm* gene. Two BAC clones amplifying the resistance allele in coupling with the *Vm* gene were isolated and the sequencing is in progress to identify candidate resistance genes.

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Poster Abstract – A.52

THE EUROPEAN SAFENUT PROJECT: AN EFFORT TO IMPROVE THE MANAGEMENT OF THE *CORYLUS AVELLANA* AND *PRUNUS DULCI* GENETIC RESOURCES

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Corylus avellana, germplasm, endangered genotypes, traditional knowledge

Corylus avellana and *Prunus dulci* are commodities of international economic importance. Different Countries conserved the hazelnut and almond genetic resources, but often the numerous local repositories are not made uniform at the European level and the problems of synonymous and mislabelling are common in the national collections. Moreover, traditional genotypes handed by father to son are conserved *on farm*. The exploitation and evaluation of such germplasm allow to recuperate the main part of the genetic variability present even at low frequencies. Within the Council Regulation (EC) N. 870/2004 AGRI GEN RES, which established a Community programme on the conservation, characterisation and utilization of genetic resources in agriculture, the project 'SAFENUT' (Safeguard of almond and hazelnut genetic resources from traditional uses to modern agro-industrial opportunities), represents an effort to coordinate the hazelnut genetic resources to share them in a more efficient manner, focussing on the recovery of old traditional endangered almond genotypes. One of the main objective of the project is the centralization of available hazelnut germplasm by harmonizing the standard descriptors for a common characterization of cultivars. Regarding *Prunus dulci*, which was one of the species considered in a previous *Prunus* AGRI GEN RES, the main goal is to recover all the local endangered varieties as well as to characterize germplasm not yet included in the reference collections. This aims at the creation of a core collection and gene banks as well as at the realization of the European virtual inventory, in order to share and spread all this information. Particular attention was paid on the cultural meaning of the genetic resources. The project benefits from the participation of a strong partnership (11 partners) from 6 European Countries, including the NGO Lega Ambiente and

Farmer Association. In the first year of the project, a survey was performed in different areas of traditional cultivation and novel hazelnut ecotypes: local almond varieties were pre-selected. SSR analysis were carried out to identify synonymous and homonyms present in the national collections. Biochemical analyses were performed on 60 genotypes. With respect to traditional knowledge, a review on the existing hazelnut exhibitions was realised and questionnaires were designed with the aim to recover historical memories on hazelnut and almond traditional knowledge and uses.

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Poster Abstract – A.53

CURRENT RESULTS ON THE LOCALIZATION AND MOLECULAR CHARACTERIZATION OF FOUR STANDS OF *AMYGDALUS WEBBII* SPACH

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Amygdalus webbbi, morphological and molecular characterization

Amygdalus webbii (Spach) Vierh. (sin. *Prunus webbii* Spach, 2n=2x=16) is a wild species found only in marginal areas and is thought to be closely related to the cultivated almond. *A. webbii* is the only wild relative of almond growing in Italy and can be found only in Apulia and Sicily, particularly at the edge of denser maquis formations.

In the present paper results on the identification, GPS localization, morphological and molecular characterization of *A. webbii* populations collected in several sites of Apulia region are reported. Four stands of *A. webbii* were identified and their stational data described.

Over twelve hundred nuts were grown, establishing a nursery in the "Martucci" experimental farm located in Valenzano (Bari). Two hundred of them were genotyped and the plantlets were characterized by means of morphological analysis. The genotypes were determined by means of SSR analyses, using primers developed on cultivated almond. These specific primers were able to amplify also wild almond DNA, thus allowing the establishment of three polymorphic descriptors for *A. webbii* SPACH.

This report is part of a larger study for the development of an integrated action (INTERREG IIIB – ARCHIMED: ECOMEMAQ) toward the sustainable development of Mediterranean areas characterized by maquis formation.

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Poster Abstract – A.54

VNTR, SSR AND MORPHOLOGICAL CHARACTERIZATION OF TOMATO ACCESSIONS SPREAD IN CAMPANIA REGION

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Lycopersicon esculentum, GATA, SSR, microsatellites, genetic diversity

In this study it has been evaluated the ability of two DNA molecular markers to discriminate a set of 16 tomato accessions. Among these accessions, some could not be distinguished on the basis of morphological traits. VNTR and SSR markers were used as a tool to characterise a tomato germplasm collection representative of Italian tomato types that are traditionally cultivated in Campania. The germplasm included material for fresh market and food-processing purposes. VNTR marker revealed that some accessions were not genetically uniform; $(GATA)_4$ fingerprinting clearly allowed the distinction of contaminating or segregating genotypes, which show different hybridization patterns. The UPGMA hierarchical classification based on a data set of 14 SSR appropriately selected, confirmed the differences observed through VNTR analysis. The genetic diversity identified in the 16 accessions revealed a consistent polymorphism at the analyzed loci, suitable to discriminate all the genotypes. Although both markers effectively discriminated the analysed samples, SSR resulted also suitable for the genetic traceability of tomato varieties along the agro-food chain.

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Poster Abstract – A.55

A COMPARISION OF MINISATELLITE AND SSR MARKERS IN RELATION TO MORPHOLOGICAL TRAITS FOR DISCRIMINATION OF 'SAN MARZANO' ACCESSION

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tomato, VNTR, SSR, microsatellites, genetic diversity

'San Marzano' is one of the most widely known tomato (*Lycopersicon esculentum* Mill.) cultivars, and is a classic example of a local variety with a premium value. Unfortunately, the original cultivated form is underrepresented in the Protected Denomination of Origin (PDO) area because of the incidence of contaminant morphologically similar genotypes. Our aim was to investigate the ability of three DNA marker systems (minisatellite, CAPS and SSR) to reveal the genetic diversity of tomato accessions that, as indicated by a morphological analysis, are phenotypically very close. The data indicate that CAPSs are the least effective, whereas both minisatellites and SSRs can be used to genetically distinguish the analysed materials and depict relationships consistent with the hierarchal pattern obtained by the morphological data. As locally cultivated tomato accessions are often characterised by some degree of genetic variability, our results will be valuable in facilitating the purification, management and breeding of tomato germplasms. The differences between the marker systems employed are discussed in relation to their usefulness in the agro-food chain.