WHAT CAN A LANDRACE CASE STUDY TELL US ABOUT ADAPTATION TRAITS?

TIRANTI B., SPATARO G., NEGRI V.

Department of Plant Biology and Agro-environmental and Animal Biotechnology, University of Perugia, Borgo XX Giugno 74, 06121 Perugia (Italy)

diversity, SSRs, outlier loci, selection, on-farm conservation

Awareness of the need for biodiversity conservation is now universally accepted. To date, conservation activities have mainly focused on ex situ and in situ conservation of wild species. However, the diversity between and within crop species also has a significant value. In domesticated crops, landraces have been, and still are, the primary source of genetic diversity for plant breeding. As such, landraces are vital genetic resources which should be maintained on farm and ex situ for future use. Few it is known about the organization of landrace diversity and about the forces acting in shaping it, although this knowledge is fundamental for breeding and conservation activities. A primary aim of this study was to obtain an insight in how variation has been built on under a cultivated environment and to identify loci that potentially underlie selective effects by using a landrace case study whose natural and human environment is known in details. Another aim of this study was to define an appropriate on farm conservation strategy for this threatened bean landrace, which can serve as a model for other threatened populations. Farmer seed lots of this landrace were examined for 18 morpho-physiological traits and 28 SSR molecular markers. Significant differences were found among them for both the morpho-physiological and molecular traits. A high level of genetic diversity and a significant genetic structure was detected among the farmer’s populations (Fst = 0.367). The landrace appears to be structured as a metapopulation in which a substantial differentiation is maintained at the subpopulation level. Evidence of locus-specific selective effects was obtained for four of the thirteen loci-differentiating subpopulations by either one of the statistical tests used (DetSel, Fdist2). Both the statistical tests showed one of these loci to be under selective pressure due to altitudinal gradient. Our data suggest that differential micro-environmental selection pressures and drift explain the observed pattern of LR diversity. An appropriate on farm conservation of a structured LR requires that subpopulations be maintained on the farms from which they come.
TUBER MELANOSPORUM GENETIC VARIABILITY AND LIFE CYCLE

RICCIONI C., BELFIORI B., PASSERI V., ARCIONI S., PAOLOCCI F., RUBINI A.

National Research Council, Plant Genetics Institute—Perugia, Via Madonna Alta 130, I-06128 Perugia, Italy

Tuber melanosporum, genetic structure, SSR markers, reproductive mode, life cycle

The growth and survival of many forest trees and shrubs depend on root colonization by ectomycorrhizal fungi. Thanks to the mycorrhizal symbiosis the host plant receives mineral nutrients while the fungus obtains photosynthetically derived carbon compounds (Harley & Smith 1983). Among ectomycorrhizal fungi those of the genus Tuber are also important for the production of highly-priced edible mushrooms (also known as truffles) with unique organoleptic qualities, such as the premium white truffles and the most appreciated black truffles, produced by the species T. magnatum Pico and T. melanosporum Vitt., respectively.

The worldwide demand for truffles greatly exceeds their natural, and dramatically declining, production (Hall et al. Trends Biotechnol 2003). Furthermore, attempts of truffle cultivation by outplanting nursery–produced mycorrhizal plants have been not always, and not for all the species, successful. It is worth to mention that key aspects of truffle life cycle such as their reproduction mode, are still under debate, since these fungi are not amenable for in vitro mating experiments and cultivation. Additionally, the ecological conditions that in each given truffle species promote fruiting are far from being fully clarified, while there are merging data supporting, at least on a large geographic range, a genetic differentiation of T. magnatum and T. melanosporum strains (Rubini et al. Appl Env Mic 2005; Murat et al. New Phytol 2006). In order to shed lights on T. melanosporum genetic variability over the entire species distributional range more than 200 samples, collected in Italy, France and Spain, were genotyped using AFLP (Amplified Fragment Polymorphism) and ITS-SNP (Internal Transcribed Spacer - Single Nucleotide Polymorphism) markers as well as T. melanosporum–specific SSR (Simple Sequence Repeats) markers identified as reported in Rubini et al. (Mol Ecol Notes, 2004). Additionally, following the procedure described in Paolocci et al. (Appl Env Mic 2006) and Rubini et al. (New Phytol 2007) these co-dominant markers were also used to check for the presence of putative heterozygous/heterokarion structures all over the different T. melanosporum life stages.

Here we report that SSR-based population genetics analysis indicates the existence of a genetic structure in T. melanosporum with the southernmost populations from Spain and Italy showing the highest levels of biodiversity, whereas AFLP identified an astonishingly high level of intrapopulation genetic diversity for a species that, up to now, has been regarded as a selfing one (Bertault et al. Nature 1998; Murat et al. New Phytol 2006). Along this line, here we provide for the first time compelling evidence that T. melanosporum outcrosses and that the haploid phase prevails in its life cycle, being the diploid/dikaryotic phase likely temporally and spatially confined in the early stages of truffle development.

In summary, we think that our study substantially deepens our understanding of T. melanosporum biology and genetic structure with profound impact for the development of strategies for the cultivation and marketing of these prestigious fungi.
ARABIDOPSIS THALIANA PLANTS OVEREXPRESSING RAMOSA1 GENE SHOW AN INCREASE IN ORGAN SIZE DUE TO CELL EXPANSION

CASSANI E.*, LANDONI M.**, BERTOLINI E.*, PANZERI D.*, PILU R.*

*) Dipartimento di Produzione Vegetale - University of Milano, Via Celoria 2, 20133 Milano, (Italy)
**) Dipartimento di Scienze Biomolecolari e Biotecnologie - University of Milano, Via Celoria 26, 20133 Milano (Italy)

ramosa1 gene, Zea mays, Arabidopsis thaliana, cell expansion, flower size

The structure of the plant inflorescence and flower is an important agronomic and ornamental trait studied for its potential economic applications. In particular, the capacity to modify flower size has always been a breeder’s goal. Genetic and molecular studies have shown that the Zea mays gene ramosa1 (ra1) is involved in inflorescence branching regulation. In fact the ra1 loss of function mutation causes extra branching of the inflorescence.

In this work we suggest a possible utilization of the ramosa1 maize gene as a tool to modify inflorescence architecture and flower size in transgenic plants. In fact overexpression of this gene in Arabidopsis plants promotes an increase in reproductive organs size. Pollen, seeds, cotyledons, leaves and roots are also larger than those of the wild type. Analysis of organs from transformants showed that cell expansion was increased without apparently affecting cell division.

The results that we obtained support the evidence for a phenomenon based mainly on cell expansion that may be mediated in some way by the up-regulation of genes involved in cell expansion regulation.

Further studies will be necessary to understand the interaction between the ra1 exogenous gene and the final target genes in Arabidopsis plants and the behaviour of the ra1 gene when overexpressed in other plant species.

Finally, it has not escaped our notice that the ra1 gene may became an useful tool for manipulations of plant size and architecture especially in ornamental plants.
AFLP in fluorescence technique is a useful tool to assess polymorphism among durum wheat genotypes. The aim of this work was to analyze and recognize by AFLP in fluorescence (fAFLP) durum wheat’s genotypes in mixture. Mixture of DNA, obtained mixing 3 known genotypes of *Triticum durum*, was analyzed with 5 primer combinations to evaluate as fAFLP technique reveals the presence of known germplasms and the method sensibility. Amplified fragments, obtained by two amplification steps - preselective and selective - was analyzed by capillary electrophoresis with genetic analysis system CEQ8000™ by Beckman & Coulter. 

Achieved results demonstrated that AFLP in fluorescence is a helpful tool to varietal identification of durum wheat as well for distinction of genotypes mixed in small percentage. These results, applied to a larger number of mixed genotypes, will concour to establish the sensibility of the method, which it could become a technique of routine’s analysis in studies of traceability of monovarietal products.
POLYMORPHISM OF B- AND C-TYPE LOW MOLECULAR WEIGHT GLUTENIN SUBUNITS IN TRITICUM TURGIDUM SSP.

URBANO M., COLAPRICO G., MARGIOTTA B.

Institute of Plant Genetics – CNR, Via Amendola 165/A, Bari (Italy) – benedetta.margiotta@igv.cnr.it

tetraploid wheat, LMW-GS, B- and C-type glutenin subunits, allelic variants

The low molecular weight glutenin subunits (LMW-GSs), play a key role in the glutenin polymer formation. They have been classified, according to their biochemical properties into B, C and D-types and on the base of their different structural characteristics as chain extender and chain terminators. The B-types include a large number of subunits forming the true LMW-GS, while the C- and D-types consist of modified monomeric proteins, similar to the so called gliadins, which can be incorporated into the glutenin polymer because they have an odd number of cysteine residues. These proteins are 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. Several studies have shown the positive effects of the LMW-GSs on gluten strength, while few works have evidentiated the influence of specific Glu-3 alleles on gluten quality, in particular of each B- and C-type glutenin subunit.

In this context, to study the polymorphism of LMW-GSs in tetraploid wheats Triticum turgidum, a collection of the subspecies ssp. turanicum, ssp. polonicum, ssp. carthlicum and a durum wheat line carrying a 1BL.1RS translocation and two Langdon D-genome disomic substitution lines 1D(1A), 1D(1B), have been analysed by one-step one-dimensional SDS-PAGE. Total proteins from different accessions and aneuploid lines have been extracted from crushed endosperm halves following fractionated precipitation with hydro-alcoholic solvent. The fractions enriched of B and C-type LMW-GSs have been subsequently separated by electrophoretic analyses and the patterns/alleles of the corresponding B- and C-types reported in diagrammatic representation. The genetic variability of these LMW-GS groups, including known alleles quality-associated, as well as the potential of novel germplasm for the improvement of wheat quality in breeding programs, will be also discussed.
ASSESSING DISTINCTIVENESS OF CROP PLANT SPECIES AND VARIETIES THROUGH DNA BARCODING

BARCACCIA G., NICOLÉ S., LUCCHIN M.

Department of Environmental Agronomy and Crop Science, Faculty of Agriculture, University of Padova, Campus of Agripolis, Viale dell’Università 16, 35020 Legnaro, Padova

genetic traceability, plastid genes, bean, corn, grape

DNA barcoding should provide an accurate and automatable method for the genetic identification of plant species and varieties by using a standardized genic or integenic region as molecular tag. While in a range of animals, the mitochondrial genes such as the cytochrome oxidase subunit I (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. Furthermore, the mitochondrial genome in plants undergoes significant rearrangement and horizontal transfer of genes, both at intra and interspecific levels. Consequently, it was suggested to use standard DNA regions from the chloroplastic genome that may offer for DNA barcoding in plants what the mitochondrial genome does for animals: it is an uniparentally inherited, nonrecombining and structurally stable genome. For phylogenetic purposes, the locus most commonly exploited in plants is that of the rbcL gene, encoding for ribulose 1,5 bisphosphate carboxylase-oxygenase. This gene was shown to be a good candidate also for genetic traceability. Some authors suggested to adopt an integrated approach of DNA barcoding consisting on the use of multiple genes for a quick and careful identification of species and varieties. Besides rbcL, other chloroplastic genes suitable for DNA barcoding include coding regions such as atpB, ndhF and matK exons and non-coding regions such as the trnL intron and the trnL-F intergenic spacer. Non-coding regions offer the advantages to have fast rates of evolution and to be short so they can be directly amplified and sequenced.

The potentials of the DNA barcoding in plants have not been investigated yet. Theoretically such strategy could be very useful also for assessing the distinctiveness of varieties and determining the relatedness among varieties of crop species, especially for mono-genotype varieties like pure lines, hybrids and clones. Our study deals with the use of DNA barcoding for the genetic recognition of plant species and varieties as well as their food derivatives. As plant materials, several pure lines of bean (Phaseolus vulgaris L.), commercial hybrids of corn (Zea mays L.) and clonal cultivars of grape (Vitis vinifera L.) were used for preliminary investigations of single gene polymorphisms in order to assess the genetic variability within species and the genetic traceability of single varieties. The three species were arbitrarily chosen because of the different reproductive and propagative mechanisms, i.e. sexual reproduction by seeds set through cross-pollination in corn and self-pollination in bean and vegetative propagation by cuttings in grape. Genomic DNA samples were isolated and purified from available plant materials of all species and characterized at the molecular level by amplifying and sequencing specific chloroplastic DNA regions, namely the rbcL gene exon, the intergenic spacer atpB-rbcL and the trnL gene intron. An extensive bioinformatic survey allowed us to preliminary retrieve nucleotide sequences of the selected
chloroplast DNA regions from the NCBI databases in the *Fabaceae*, *Poaceae* and *Vitaceae* botanic families: 216, 161 and 26 entries for rbcL; 38, 50 and 28 entries for atpB-rbcL; 151, 103 and 36 entries for trnL, respectively. After serial local multiple sequence alignments, specific primer pairs were designed in highly conserved short stretches flanking the most variable regions in order to clone the orthologous sequences in bean, corn and grape species. The research is in progress with the main goal of discovering sequence-specific SNPs and haplotypes to be exploited for the precise identification of species and varieties.
FLC AND THE REGULATION OF FLOWERING TIME IN CHICORY

LOCASCIO A., VANNOZZI A., PARRINI P., LUCCHIN M., VAROTTO S.

Department of environmental agronomy and crop production, University of Padova – Agripolis, Viale dell’Università 16, 35020 Legnaro (PD)

vernalization, Chicorium intybus, RNAi, FISH, ploidy

The transition from vegetative to reproductive development for a plant is a highly regulated process sensitive to environmental cues, as day length and temperature. Winters annuals and biennales typically require a prolonged exposure to cold to flower in the spring. The process by which the meristem gains the competence to flower after the experience of low temperatures is known as vernalization. In the model plant Arabidopsis thaliana, the ability to flower is related with the silencing of a floral repressor named Flowering Locus C. FLC is negatively regulated by vernalization, instead its expression is inducted by the gene FRIGIDA. The silencing of FLC is an epigenetic process, mitotically stable, but it seems reset after meiosis. Up to now FLC has been isolated only from species belonging to Brassicaceae family and from sugar beet.

Wild chicory (Cichorium intybus L.) is a biennial species, belonging to Asteraceae family. Chicory is a crop mainly cultivated in North Eastern of Italy and it shows a quite obligate request of cold to flower.

In our study, we are investigating the molecular bases that regulate the flowering in chicory by vernalization.

We isolated FLC homologues from chicory and characterized their expression patterns in plant tissues and in response to vernalization. Given the similarities of sequence, pattern of expression and localization of FLC observed between arabidopsis and chicory, a construct 35S::FLC was made to transform the mutant flc-3 of arabidopsis var. Columbia, with the purpose to complement the repressive mutation flc-3 and rescue the phenotype.

Furthermore the knock-down of FLC in chicory could be useful to verify if FLC is involved in the process of flowering repression mediated by vernalization as in Arabidopsis. RNA interference mediated by miRNA could be the strategy to induce the silencing of FLC. A specific construct to induce interfering was produced and the transformation of chicory has been carried out through Agrobacterium infection of leaf disks. Plant regeneration via organogenesis will be achieved and the selection of the transgenic plants carried out.

In Italy, different types of chicory were selected by breeders and these types show quite different flowering time, as well as morphological differences among them and from the wild chicory. Fluorescent In Situ Hybridization on chromosomes (FISH) will be used for checking the possibility of events of aneuploidy or polyploidy in the cultivated varieties. The investigations on ploidy level would also explain why the number of transcripts identified in the cultivar Treviso differed from the number identified in the wild type chicory.
DEVELOPMENT OF A DArT MICROARRAY FOR COMPARATIVE STRUCTURAL GENOMICS AND MAPPING OF AGRICULTURALLY SIGNIFICANT GENES IN WILD POTATO SPECIES

IORIZZO M.*, BRADEEN J. M.**, AVERSANO R.*, CARPUTO D.*

*) Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples “Federico II” (Italy)
**) Department of Plant Pathology, University of Minnesota (MN, USA)

wild potato species, DArT, comparative structural genomics

Efficient access to genetic variability is important for breeding programs. For potato improvement, the approximately 180 wild species represent a valuable source for agriculturally significant genes, including genes for disease resistance and cold tolerance. We specialize in a group of 20 wild potato species that collectively comprise the tertiary genepool for cultivated potato. These species are sexually incompatible with cultivated potato, but genes from these species can be accessed using bridge crosses, somatic hybridization, and gene cloning and transformation. To improve access to agriculturally significant genes from tertiary genepool species, we have initiated an effort of comparative structural genomics using the Diversity Array Technology (DArT) marker platform. The first phase of this project was development of the DArT microarray. We used five diverse tertiary genepool species for array construction: *Solanum commersonii*, *S. bulbocastanum*, *S. polyadenium*, *S. chacoense*, and *S. pinnatisectum*. Six accessions for each species were included. Now, DArT array validation via phylogenetic comparison is ongoing. In this phase of our study, deduced relationships between the five species used in array construction plus *S. circaeifolium* and *S. cardiophyllum* will be compared with those reported previously based on morphological and molecular markers. Congruence between the DArT phylogeny and previously reported phylogenies (an expected result) will be interpreted as validation of the DArT array. For *S. commersonii*, *S. bulbocastanum*, *S. chacoense*, and *S. pinnatisectum*, parental genotypes have been designated based on crossability studies and preliminary phenotypic evaluations conducted by our laboratories. F1 mapping populations have been generated for each species. Linkage maps will be constructed for each species based on a common set of DArT markers, allowing comparison of genome structures. Significantly, the maps generated will allow efficient mapping of genes conditioning agriculturally significant phenotypes. In support of this effort, large-scale phenotypic analyses are ongoing. Currently phenotypic tests include evaluation of all available accessions of *S. commersonii* (133 accessions) and *S. chacoense* (61 accessions) for foliar resistance to *Phytophthora infestans*. 
ANCHORING OF *SOLANUM HABROCHAITES* (ACC. LA1777) ILs TO A WHOLE GENOME PCR-BASED MAP AND IDENTIFICATION OF QTL FOR AGRONOMIC TRAITS

TRIPODI P.*, FRUSCIANTE L.**, TANKSLEY STEVEN D.***, GRANDILLO S*

*) CNR-IGV Institute of Plant Genetics, Portici, Via Università 133, 80055 Portici, (Italy)
**) Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples “Federico II”, Via Università 100, 80055 Portici (Italy)
***) Departement of Plant Breeding and Genetics, Cornell University, 14853 Ithaca, NY (USA)

wild species, *Solanum habrochaites*, introgression lines, molecular markers, QTL.

Populations of introgression lines (ILs) or “Exotic libraries”, which represent the entire genome of a wild species, have proven to be very efficient tools for exploring and using the hidden breeding potential of unadapted germplasm to improve the performance of elite genotypes (Zamir 2001; Gur and Zamir 2004).

In tomato, a population of *Solanum habrochaites* (acc. LA1777) (H) ILs in the genetic background of *S. lycopersicum* (cv. E6203) was developed by Monforte and Tanksley (2000). The population provides a coverage of approximately 85% of the wild genome, and some of the lines still contain multiple wild introgressions. In order to increase the mapping resolution and the wild genome coverage of this population, a new set of H ILs and sub-ILs containing single wild introgressions is being developed. For this purpose BC1 and BC2 populations have also been analyzed. Furthermore, the H IL population is being anchored to a PCR marker based framework which will facilitate QTL identification, mapping, cloning of the underlying genes and the use of the novel variation in marker-assisted breeding. A set of the H ILs have been used to identify QTL for traits of agronomic interest including size, shape, colour, and soluble solids content of the fruits.
The crimson clover (*T. incarnatum* L) presents a narrow natural genetic variability that limited the possibilities to select new varieties. With the view to increase the genetic variability, in 1999 it has been initiated a program of experimental mutagenesi using as initial population the variety "LIDIA", and as chemical mutagen agent, the 2,4 DB [4(2,4 dichlorophenoxies) butiric acid].

In the Mo generation, except the unfavorable mutagen forms (dwarf plants, plants with partial or total chlorophylian deficiencies, very precocious or plants without any agronomic value, etc.), with an extremely limited frequency (0,8 -1,2 x 10^-6) has been identified a plant having a later vegetation period and that presented sectorial chimeras for the color flower on the same inflorescence (red and white). This new mutant, conventionally denominated EG-01-MLI, at the beginning of the flowering time has been isolated and forced to selfpolinated. From the seeds picked up from this plant, in the M1 generation has been gotten plants with the color flowers completely red (EG-01-MLI-R) and plants with the color of the flowers completely white (EG-01-MLI-w) in a proportion more or less of 1:1. Each one of the two populations in the generation M2 has still been forced to selfpolinated. In the next generation (M3) from the plants having the inflorescence with white color of the flowers it have been gotten only plants with the inflorescence with white color of the flowers, while from the plants with the inflorescence with red color of the flowers it have been gotten plants with the inflorescence with red color of the flowers, plants with the inflorescences with pink color of the flowers and plants with the inflorescence having white color of the flowers in a proportion of almost 2:1:1.

Even, presently there are carrying out studies directed to establish the genetic control of the color flower of the mutant EG-01-MLI, the results already gotten, suggested us that the color flower of the mutant EG-01-MLI could be under the genetic control of two independent genes with cumulative effect. In the same time, by means of an individual selection practiced for many generations both for forage value and for seed production conducted in more environments of Italy, it has allowed us to get a new crimson clover variety denominated "Snow’s Ball" variety of an elevated agronomic interest, characterised by a middle-late vegetation period and with white color of the flowers. Comparing others varieties, this last characteristic represents a novelty for the National Register of Agrarian Varieties.
CANDIDATE GENE-BASED ASSOCIATION STUDIES IN GRAPEVINE: THE BERRY SIZE TRAIT


*) Department of Genetics and Molecular Biology, IASMA research centre, Via Mach 1, I-38010 San Michele a/Adige (Italy)
**) UMR 1097 Diversité et adaptation des plantes cultivées, INRA-SupAgro - IRD, 2 place P. Viala F-34060, Montpellier (France)
***) UMR Sciences pour l'oenologie, INRA-SupAgro, 2 place P. Viala F-34060, Montpellier (France)

association studies, linkage disequilibrium, SNPs, berry size

Since in Grapevine most traits of economical and agronomic interest have quantitative nature, QTL (Quantitative Trait Loci) analysis are necessary. A new concept based on linkage disequilibrium (LD) has recently been adapted for searching QTLs. Association analysis (LD mapping) has been used in plants and has also been applied to the characterization of a simple trait in grape. In this study, we performed a candidate gene-based association analysis in order to characterise the genetic determinism of berry size, an important trait linked to the final quality of wines. The program of berry development is strongly and irreversibly impaired by changes in the microenvironment between anthesis and véraison. Previous microarray analysis and cDNA suppressive subtractive hybridisation led to target VvHB13 as a key homeotic gene strongly associated with flesh development at green stage. The ORF and 3’UTR were complete on the Syrah cDNA clone and 1.5 kb from 5’-end to the ATG were sequenced on a Cabernet Sauvignon BAC clone of INRA-Evry, before additional 4.4 kb till the previous transcript were obtained by blastN on the Pinot Noir assembled genome available at IASMA. VvBURP1 appeared as another candidate gene in the same study on the consequences of the fleshless mutation on young berry transcriptome; its promoter and 3’UTR were isolated thanks to the alignment of the coding sequence and the assembled genome sequence of Pinot Noir. Both full-length genes were then sequenced in a core-collection of 141 V. vinifera genotypes defined on the basis of agronomical and morphological data including berry size from the Vassal collection of INRA-Montpellier. Based on an unified mixed-model, taking into account the structure and the kinship within the collection, SNPs (Single Nucleotide Polymorphisms) significantly associated to the berry size trait were identified and are reported.
MITOCHONDRIAL DNA BARCODING AS A TOOL FOR THE GENETIC TRACEABILITY OF FISHERY DERIVATIVES

NICOLÉ S., ECCHER G., BARACCIA G.

Department of Environmental Agronomy and Crop Science, Faculty of Agriculture, University of Padova, Campus of Agripolis, Viale dell’Università 16, 35020 Legnaro (Italy)

mtDNA barcoding, COI, CytB, fishes, species identification

DNA barcoding is a technique for characterizing species of organisms using a short DNA sequence from a gene of known function and position in the genome. The core idea of this technique is that nucleotide variation for short pieces of DNA can be mostly found between species and only to very low extents among organisms within species. DNA markers suitable for genetic traceability purposes usually belong to the mitochondrial genome because of its haploid nature, maternal inheritance, and multiple copies in the cell. COI (Cytochrome oxydase subunit I) was originally used as specific mitochondrial gene for DNA barcoding: a 648 nucleotide long sequence was selected near to the 5’ end of the gene with two conserved flanking sites in most animal groups where universal primers were then designed. These primers supplied very reliable results in all taxa tested so far and they also enabled the recovery of gene polymorphisms for most animal phyla. Besides, the evolution of COI is rapid enough, because of the high incidence of base substitutions in third-position nucleotides, to allow the discrimination of closely related species and sometimes phylogeographic groups within a single species. Several studies have now established that sequence diversity in this portion of COI provides strong resolution at the species level for several animal groups including birds and fishes.

The aim of our research is to apply the DNA barcoding as an helpful strategy for genetic traceability of marine species and their food derivatives, with particular reference to three different taxonomic groups: fishes, molluscs and shellfishes. According to the criticisms recently provided by scientific community supporting the theory that a single gene may not be sufficient to univocally identify a species, in addition to the Cytochrome oxydase subunit I (COI) we have selected other two mitochondrial genes encoding for Cytochrome b (Cyt b) and ribosomal RNA small subunit (16S-RNA). After the selection of species on the basis of their economic relevance, the experimental steps adopted for the in silico analyses were the following: i) retrieval of mitochondrial sequences from NCBI nucleotide databases for each of the selected genes; ii) removal of redundant and unreliable entries, and editing of sequences; iii) evaluation of intra- and inter-specific polymorphisms (SNPs and In/Dels) by means of multiple alignments; iv) design of forward and reverse primers on highly conserved regions of the consensus sequences for each gene and group of species belonging to the same family. We are now performing serial in vivo analyses using genomic DNA samples isolated from commercial food products containing one or more fish, mollusc and shellfish species. A number of DNA samples from pure species for each family were also included as reference standards. The experimental steps currently adopted for the identification of species are the following: i) amplification of the target sequences using the specific primer pairs; ii) subcloning and sequencing of the PCR products; iii) Blast analysis against the non-redundant nucleotide databases using each of the sequences as query; iv) computation of the substitution
matrices and identity indices for species identification. The research is in progress with the main goal of setting a multilocus genetic traceability system, assessing the organism taxonomic identity and verifying the food product label information.


**TRITICUM TURGIDUM VAR. DICOCCUM AS SOURCE OF POWDERY MILDEW RESISTANCE GENES**

SIMEONE R., MANGINI G., GADALETA A., BLANCO A.

Department of Environmental and Agro-Forestry Biology and Chemistry, University of Bari, Via Amendola 165/A, 70126 Bari (Italy)

tetraploid wheat, powdery mildew resistance, microsatellites

Powdery mildew, caused by *Erysiphe graminis* f. sp. *tritici*, is one of the devastating wheat diseases in areas with temperate climates. Breeding for varieties with resistance alleles is the most economical and effective way for controlling the disease. To date, 36 gene loci for resistance to powdery mildew were discovered and assigned to specific chromosomes of wheat. However, due to the pathogen evolution new sources of resistance are constantly required for a genetic control of this disease. *Triticum turgidum* var. *dicoccum*, a primitive form of cultivated emmer, is a good source of powdery mildew resistance. Previous germplasm screening identified the accession MG5323 to be resistant to natural populations of powdery mildew.

The accession MG5323 was crossed to *Triticum turgidum* var. *durum* cv. Latino, susceptible to powdery mildew. A set of 120 recombinant inbred lines (RILs) was produced by single seed descent. RILs and parental lines were tested for resistance to natural mildew population. The analysis showed 99 susceptible lines (score 2-4) and 21 resistant (score 0-1). The chi-square test fitted to a 3:1 segregation ratio in the RIL population, thus suggesting the presence of two recessive genes in the var. *dicoccum*. Bulked segregant analysis (BSA) performed with microsatellites markers (SSR). Four markers were found to be polymorphic in the two bulks. Such markers were screened on the complete set of RILs. The regression analysis showed a highly significant association of three markers (*Xwmc25*-2B, *Xwmc243*-2B and *Xwmc257*-2B) located on short arm of chromosome 2B.

In order to identify the second locus controlling the resistance to powdery mildew, a cross between two RI lines having the 2B resistance gene but different for the reaction to powdery mildew was made. The F$_2$ population was tested for resistance to powdery mildew and molecular analysis was performed by using BSA.
DEVELOPING ITALIAN RICE VARIETIES FOR CULTIVATION IN SIMIL-UPLAND CONDITIONS

GREPPI D., CAVIGIOLO S., LANZANOVA C., LUPOTTO E.

C.R.A- Istituto Sperimentale per la Cerealicoltura – Sezione specializzata per la Risicoltura-Vercelli (Italy)

water shortage, plant performance, WUE, quality traits

Rice (*Oryzae sativa* L.) is the most diverse crop in the world and it can be grown under irrigated (lowland) or rainfed (upland or lowland) conditions. Rainfed rice occupies about 45% of the global rice area and accounts for about 25% of the rice production. During the last years in Italy, water shortage occurring early in the cropping season represents an important problem for rice production, and therefore the identification of varieties which adapt better to sudden water deficit is a major requirement. The major objective of this research is to evaluate the performance of Italian rice cultivars under simil-upland conditions with turnated irrigation, in comparison with the flooded system, as far as the main agronomical, phitosanitary and quality traits are concerned.

The field experiments were conducted throughout two years (2004-2005) at C.R.A.- Rice Research Section, in Vercelli, Italy. During the cropping season, in the experimental field under similar dry conditions, three irrigation treatments by flushing method were performed. Total water supply (rainfall plus irrigation) was 3491 m$^3$/ha in 2004 and 3907 m$^3$/ha in 2005. Water Use Efficiency (WUE) representing the quantity of paddy rice produced (kg ha$^{-1}$) for mm of total water used., was calculated. This study showed that, when compared to the flooded system, the water shortage caused an average 43% yield reduction, which was the result of a variation of the grain yield components (i.e. panicle sterility +54%, tiller density -34% and 1000 seed weight -19%). However, under water shortage conditions, the highest yields were obtained by Eurosis (6.07 t/ha), Augusto (5.50 t/ha) and SIS R215 (5.47 t/ha), which showed the best WUE value as well. The results related to quality traits indicated that water shortage caused a reduction in the milling grain, with the cv. SIS R215 being the less affected. In addition, the amylose content increased significantly in some varieties (Eurosis and Gange) if compared to the flooded system. The culture morpho-phenological evaluation showed an average 20% increase in the cultural cycle and an average 20% decrease in the plant height. Although the infection degree of the seed increased under the simil-upland system, if compared to the conventional culture, it did not correlate with presence of any important mycotoxin (DON, ZEN, FB1).

The study is performed within the framework of the Eu-funded project CEDROME (INCO-CT2005-015468); M.V. holds a grant of the Italian MIPAAF.
RI-DOMESTICATION OF BLUE LUPINE (*LUPINUS ANGUSTIFOLIUS*) ADAPTED TO CULTIVATION IN ITALIAN SUB ACID SOILS

BOZZINI A.*, CHIARETTI D.**, STAMIGNA C.*

*) Proseme, Enna. (Italy)
**) Biotec, Casaccia, ENEA. Roma

sweet blue lupine, domestication, breeding

*Lupinus angustifolius* L. (blue lupine) is a wild Mediterranean species growing profusely in acid and sub acid, sandy or volcanic soils, particularly of Tyrrhenian coastal areas of Italy. These wild types are also well adapted to poor, marginal soils, often used only as pastures for ruminants.

A breeding programme has been developed to select domesticated lines by transferring domestication characters present in cultivars bred in Australia and Poland into three Italian wild selections, highly adapted to central and south Italy pedoclimatic conditions and highly productive. Crosses were made and selection performed for the several domestication genetic characters desired. Results of the research, related to potential sweet seed production in intensive cultivation and to the main domestication characteristics of the new selections, are reported.
In organic agriculture, seeds must be organic and in organic seed crops of lucerne, weeds and diseases have a key role in limiting suitable environmental conditions, by increasing the production costs and often not ensuring standard seed quality. The effect of row spacing (30 and 50 cm) and seed density (300, 600 and 900 seeds m\(^{-2}\), equivalent to 5, 10 and 15 kg of seeds ha\(^{-1}\)) on seed yield and its components (number of fertile stems, number of inflorescences per stem, flowers per raceme, pod set, seed per pod, seed set) of an organic seed crop of lucerne were assessed in a split-plot experimental design with four replicates, with row spacing as main plot and seed density as split plot. The experiment includes also a control treatment based on 900 seeds m\(^{-2}\) sown in paired rows, at 12 cm within pair and 70 cm between pairs. The experiment comprised three lucerne varieties, chosen on purpose with contrasting size of their genetic base: Ecotipo Romagnolo (with the largest genetic base), Cuore Verde (a variety registered for organic agriculture and intermediate), and Syn-2 (a syntetic derived by intercrossing 10 genotypes). The results indicate that in a specialized, organic seed crop of lucerne, the increased sowing density from 5 to 15 kg ha\(^{-1}\), greatly improves the crop competitiveness within the row against weeds; weeds presents between rows are easily controlled by split-hoe cultivation. Seed yields in 2006 were generally low and some of the seed yield components indicate that the narrowest row spacing and the low sowing densities enhances the number of reproductive stems and the total number of inflorescences m\(^{-2}\). The three varieties responded in different way to row spacing and sowing density in terms of number of flowers m\(^{-2}\) and other seed yield components. The realized seed yields were only 1% of the potential yields estimated through the available ovules per unit area.
An interest in lucerne for pastures has recently arisen, in parallel with a greater concern for a sustainable development in agriculture and a better profitability of marginal areas, where little agronomic alternative exists to grazing of sheep or adapted cattle. Grazing can be a strategic exploitation of forage resources in particular environments and livestock systems, and lucerne may represent an important tool available to producers, owing to the positive attributes possessed by this legume species. This is particularly relevant to farms with organic livestock production, where options for grazing are of paramount importance. The major constraint to an increasing development of lucerne pastures has been represented by the poor persistence of traditional cultivars under common grazing systems. In recent years, a better understanding has been achieved of morphological and physiological mechanisms underlying the tolerance to grazing of lucerne, such as the presence of a deep-set crown, a decumbent growth habit, the ability to accumulate underground reserves, and a sideways spreading ability through creeping roots or underground rhizomes. While starting this selection programme, a large germplasm collection of the *M. sativa* complex was assembled and evaluated for morpho-physiological features. Unlike other breeding programmes, we attempted to categorise the observed plants into distinct "models", based on their morphology and vigour, and we definitely distinguished between creeping-rooted and rhizomatous plants. Genotypes preliminarily selected for their positive features were polycrossed "by model" and entered the next phase of selection. Given the outstanding difficulty of fixing the creeping-rooted character, due to the complex genetic control of this trait, a parallel activity is being carried out, aiming at selecting molecular markers associated to the character, to be used in the marker-assisted selection of creeping-rootedness in lucerne. Meanwhile, rhizomatous progenies belonging to different models, with habit varying from prostrate to semi-erect and vigour from low to moderately high, were evaluated in Lodi, northern Italy, under actual grazing by sheep. Continuous stocking and intensive grazing were applied, as recommended for an effective screening and selection for grazing tolerance by the North American Alfalfa Improvement Conference (NAAIC) standard test. After a two-year assessment of grazing tolerance with reference to the behaviour of a tolerant and an intolerant check variety, the most promising germplasm was selected and assembled into six experimental cultivars (EC), three of which with narrow genetic base and three with broad genetic base. One EC has prostrate habit, three have semi-prostrate habit, and two have semi-erect habit. The six EC were further evaluated for two years under similar conditions of continuous stocking and intensive grazing by sheep. Five of the six EC showed a final persistence similar to, or better than, that of the tolerant check variety. In particular, the prostrate EC ( provisionally termed ‘Camporegio’) had outstanding persistence. Another, semi-erect EC ( provisionally termed ‘Verbena’) emerged from this evaluation work, possessing a good balance between grazing tolerance, potential dry-matter yield and seed yield (these yields being assessed in separate plots).
After the necessary steps, this germplasm is meant to be released as the first case of grazing-tolerant lucerne cultivars selected in Italy.
CHARACTERIZATION OF DERIVATIVES FROM 
*M. SATIVA X M. ARBOREA HYBRIDIZATION*

BIAZZI E., DEPEDRO C., TAVA A., ODOARDI M., CARELLI M.
C.R.A Istituto Sperimentale per le Colture Foraggere, Viale Piacenza 29, 26900 Lodi (Italy)

interspecific hybridization, saponin, flower colour

The plant material studied has been obtained by E. T. Bingham (University of Wisconsin, Madison, WI) by hybridization of the male sterile clone of *Medicago sativa* (Magnum III x Blazer XL) and *M. arborea* (Bingham and Haas, 2005). F1 plants (ten individuals) were crossed to *M. sativa* non dormant cv. Sequel, giving rise to Sativa Arborea Cross (SAC) Derivatives.

Two of the SAC derivatives (SAC 9 and SAC 10, represented by 73 and 46 plants respectively) together with (Magnum x Blazer) x Sequel parent (23 plants) were transplanted individually in soil filled-tubes 5 cm diameter at Lodi Institute in September 2005 in an open greenhouse. The aim was to characterize SAC derivatives for bio-agronomic traits and saponin composition in order to identify possible new variation coming from the integration of part of *M. arborea* genome in *M. sativa* background.

The traits considered referred to winter survival, dry matter production, earliness and plant form (number of stems and height of the five main stems) along three cuttings and pod fertility in crossing. As *M. sativa* and *M. arborea* have respectively purple and yellow flowers, the colour of flowers was used for the estimate of hybrid state, following a scale for visually scoring proposed by D. K. Barnes. Chemical analyses of stems and leaves were performed in the second cut.

The variation in saponin composition was assayed by TLC on individual plant basis in the third productive cycle; twelve individuals were selected as representative of the different TLC patterns obtained and the stability of the patterns over seasons was checked. The GC-FID analysis of the sapogenins showed an important variation for medicagenic (0.16 – 9.78 mg/g) and zanhic acid (0.39 – 2.54 mg/g).

*M. arborea* parental clones and selfed S1 progenies (67 plants representing 8 families of SAC 9 population, 102 plants representing 7 families of SAC 10 population and 8 plants belonging to two families of MB parental population) are currently under study, in order to investigate the segregation of the following characters: flower colour, saponin content and composition, capacity of regrowth after cutting, presence of foliar and stem diseases.
GENETICS AND BREEDING OF NEMATODE RESISTANCE IN
PHASEOLUS VULGARIS L.

CARBONI A.*, DEL BIANCO F.*, PARISI B.*, RANALLI P.*, DI VITO M.**

*) CRA-Istituto Sperimentale per le Colture Industriali, Via di Corticella 133, 40128 Bologna (Italy)
**) Istituto per la Protezione delle Piante - CNR, Bari (Italy)

common bean, Meloidogyne spp., resistance, SCAR markers, breeding

The search for innovative strategies able to describe new explanatory hypothesis is a fascinating challenge in the genetic contemporary era.

New techniques, emerging tools and tips, an impressive amount of information are becoming available every day, more and more.

Sometimes these new “omics” opportunities seem too fast emerging to be completely taken advantage of but never like today researchers are so free and encouraged to walk (or run) through a genome using different approaches, to test different assumptions and to conjugate Genetics with other biological matters.

The experience done by our group on this area could be defined as paradigmatic, since we were able to focus our attention and efforts on an almost new topic, the resistance of root-knot nematodes (Meloidogyne spp.) in common bean, using an innovative approach which was the sum of several traditional and pioneering methodologies.

When we started this project there were only 2 varieties registered in the world (inadequate for the European market), and in the literature a confused description of the genetic control of the resistance and no more.

Our work relied on:
1) a new phenotypic characterization;
2) a new breeding program and the development of segregating progenies;
3) a proved description of the genetic control of this resistance;
4) an innovative genomic and evolutionary study on 7 different genotypes representative of the 2 geographically distinct common bean genepools (Mesoamerica and the Southern Andes); this walk through their genome was carried out using a conserved domain of the NBS-LRR gene family as genomic marker, which resulted able to describe and measure the evolution of this resistance family in this species;
5) finally, the original intuition to link the sequencing program results, 176 new Resistance Genes Analogs were discovered, with the pedigree of a resistant accession and a derived cultivar. This “genomic pedigree” intuition let us the opportunity to describe a new SCAR marker which resulted 100% associated to the resistance.

With this new marker we were able to speed up the selection among the large amount of breeding lines we created in the Breeding program and today to present “Arechi”, the first Italian cultivar resistant to Meloidogyne incognita race 1 and Meloidogyne javanica; but also, this marker was able to complete an evolutionary journey through 250 wild accessions of common bean coming
from both genepools and to describe new hypothesis on the evolution and the dynamics of resistance genes in *Phaseolus vulgaris* L. and in the Leguminous family.
PERFORMANCE OF TOMATO PARTHENOCARPIC LINES IN EARLY GREENHOUSE CULTIVATION IN CENTRAL ITALY

OLIMPIERI I.*, MOSCONI P.*, SANAMPUDI V.R.R.*, SCHIAPPA A.**, MAZZUCATO A.*

*) Department of Agrobiology and Agrochemistry, University of Tuscia, Via S.C. de Lellis snc, 01100 Viterbo (Italy)
**) Enza Zaden Italia srl, S.S. Aurelia Km 96,710, 01016 Tarquinia (Italy)

fruit set, parthenocarpy, pat-2, tomato

The term “parthenocarpy” was introduced to indicate the formation of seedless fruits in the absence of functional pollination or other stimulation. Parthenocarpy occurs in many species, especially in several important horticultural crops and among these in tomato. Seedlessness is a desirable commodity for consumers, as well as a useful trait for genetic improvement since it would allow good fruit set in environments unfavorable for pollination and fertilization. Moreover, the knowledge of the genetic and physiological mechanisms underlying parthenocarpy represents an interesting challenge for scientific research. In tomato, different genes able to confer parthenocarpic have been described; among them, the parthenocarpic fruit-2 (pat-2) mutation deserves particular interest because it drives the formation of fruits of a size comparable to that achieved by seeded fruit in normal near isogenic lines (NILs). However, the adoption of this trait in breeding tomato varieties and hybrids has been hindered by unwanted pleiotropic effects and the lack of molecular tools for assisted selection.

The aim of the present work was to assess the performance of parthenocarpic pat-2 lines in different genetic backgrounds and in comparison with the respective normal NILs. Four pairs of wild-type (WT) and pat-2 lines in the background of cvs. Super Marmande, Early Mech, Porphyre and Monalbo were raised from February to May in greenhouse conditions in the coastal area of Latium. At harvest, significant differences were recorded for all characters observed except than for 1000 seeds weight and total soluble solids (TSS). Compared to the WTs, the parthenocarpic lines had a significantly higher number of fruits at the first harvest and mean fruit weight. When data were taken on 10 selected fruits, no significant difference in size and shape was observed between Pat-2 and pat-2 lines. For the precocity index, number of seeds per fruit and fruit firmness, significant genotype × parthenocarpy interactions were detected. In conclusion, the general thought that pat-2 entails a higher TSS value and reduced firmness seems to be related to specific genetic backgrounds.

To pursue the mapping of the Pat-2 locus, 150 F2 plants segregating after the cross between Solanum lycopersicum cv Severianin and S. pennellii LA716 were raised in the same conditions. F2 plants were largely unfruitful due to the general condition of exerted stigma and the pat-2 mutation showed low expressivity. However, it was possible to classify with certainty a suitable number of recessive plants in order to compose contrasting DNA pools to use in bulk segregant analysis.
Isatis tinctoria, mating system, setting, inbreeding depression, breeding

*Isatis tinctoria* L. (woad) is a tetraploid outbreeding biennial (*2n* = 4*x* = 28) belonging to the family of *Cruciferae*. The species was cultivated in ancient time to produce indigo, a natural blue pigment used principally for dyestuff. Nowadays the interest in cultivation of this ancient dye plant is increasing because of its adaptability to marginal conditions and increasing demand for natural products. Presently woad crops are planted with seed of natural populations. Reintroduction will be facilitated by the use of varieties bred for each proposed environment. Breeding woad requires knowledge of the mating system which is not available. In order to assess the reproductive behaviour of *I. tinctoria*, setting and progeny plant vigour were evaluated under different mating conditions.

**Evaluation of setting** - In spring 2002, 56 single plants of the population CR 2169/99 (Switzerland) were transplanted in the field. Before scape emission 18 plants were isolated singularly with micro-perforated, 18 were divided into 3 groups of six plants isolated with a bag each and 20 plants were not isolated (outcrossing conditions). At harvest, the fruit yield, the weight of a singular fruit and the seed germinability were recorded on single plants. A one-way analysis of variance (ANOVA) to test the “mating system” source of variation was used to work out results.

**Evaluation of progeny** – In spring 2004, 16 self-pollinated lines, 3 controlled-cross lines and 20 outcrossing lines derived from the previous experiment were used to evaluate the progeny performance and inbreeding depression. Twenty-one plants per each line were arranged in the field in a randomized complete block design with three replications. Progeny vigour was recorded per plant with reference to the rosette diameter and to the height of each plant.

Data were analyzed using an ANOVA where the “among progeny” source of variation was partitioned as “type of mating”, “among lines from selfing”, “among lines from controlled crossed” and “among lines from outcrossing”. The significance of each source of variation was tested over the error.

Results showed the existence of an outcrossing system in *I. tinctoria*. Obligate self-pollinated plants produced fewer siliques with lower weight and lower seed germinability than outcrossing plants. Self-pollinated progenies also generally showed lower vigour than outcrossing progenies.
DEVELOPMENT OF AN ON-LINE DATABASE OF MOLECULAR AND PHENOTYPIC DATA FOR MARKER ASSISTED SELECTION OF CEREALS


*) Department of Agricultural and Food Sciences, University of Modena and Reggio Emilia, Via J.F. Kennedy 17/19, 42100 Reggio Emilia (Italy)
**) Information Engineering Department, University of Modena and Reggio Emilia, Via Vignolese 905, 41100 Modena (Italy)

database, MAS, cereal, assisted breeding

The CEREALAB database; an information system for breeders is a source of molecular and phenotypic data, realized by integrating two already existing web databases, Gramene and Graingenes together with the source storing the information achieved by research groups of the CEREALAB project. The new data derives from a systematic genotyping work using already known markers and some brandly new protocols developed by the discovery workpackage of the project.

This integration is obtained using the MOMIS system (Mediator Environment for Multiple Information Sources). The result obtained is a queriable virtual view that integrates the three sources and allows performing selection of cultivars of barley, wheat and rice based on molecular data and phenotypic traits, regardless of the specific languages of the three source databases.

The phenotypic characters to be included in the database have been chosen among those of major interest for the breeders and divided into six categories: Abiotic Stress, Biotic Stress, Growth and Development, Quality and Yield. As far as molecular data is concerned the major categories for the query are: Trait, Qtl, Gene and Marker.
ANALYSIS OF GENOTYPE-BY-ENVIRONMENT INTERACTION IN WHEAT USING ANEUPLOID LINES WITH CHROMATIN INTROGRESSED FROM DASYPYRUM VILLOSUM

VACCINO P.*, CORBELLINI M.*, CATTANEO M.*, NEGRI S.*, PASQUINI M.*,,
CIONINI P.G.***, CACERES E.***, VITTORI D.****, CIOFO A.****, DE PACE C.****

*) CRA-Experimental Institute for Cereal Research, Via R. Forlani 3, 26866 Sant’Angelo Lodigiano, Lodi (Italy)
**) CRA-Experimental Institute for Cereal Research, Via Cassia 176, 00191 Roma (Italy)
***) Department of Cellular and Environmental Biology, University of Perugia, Via Elce di Sotto, 06123 Perugia (Italy)
****) Department of Agrobiology and Agrochemistry, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo (Italy)

GxE interaction, breeding lines, aneuploid lines, Dasypyrum villosum, low-input farming system

The presence of genotype-by-environment interaction (GxE) impose additional efforts in selection of superior breeding lines, and an understanding of the type of GxE interaction is important in all stages of plant breeding especially for pursuing sustainable low-input agriculture aims. Crop production in low-input agriculture requires cultivated varieties showing resilience (low phenotypic plasticity or high stability over environments) to varying environmental effects in farmers’ fields. Breeding methods for low phenotypic plasticity require the assessment of the genotype x environment interaction pattern for both parental lines and selected progenies, and have an important bearing on usefulness of the resulting cultivated varieties. The most important genotype x environment interaction patterns in breeding materials can be divided in two categories: one of low genotypic variance, significant crossover interaction and genotypes endowed with environmental specialization, and the other that leads few genotypes to perform better than others in several environments (“universal” genotype).

Twelve inbred breeding lines (IBLs) derived from interspecific hybridization of Triticum aestivum cv “Chinese Spring” (CS) x D. villosum (Dv) were used to explore which genotype x environment interaction pattern will be expressed when tested in multienvironment low-input cropping system. Those lines have been selected from a population of 150 aneuploid lines developed through [(CS x Dv, F1) x CS] backcross, followed by three generation of selfing (BC1F1 S1 through S5), five generations of single-spike descent (from S4 through S8) and four generations of seed increase (S12 IBLs). S12 breeding lines traced to the same S4 plant were considered “sister lines”. Genetic uniformity within lines an differentiation among lines have been tested using AFLP and GISH. The lines have been tested in 7 different environments. In each cropping environment, off-farm inputs were minimized. The main genotype x environment interaction pattern displayed by the breeding lines is of the category “higher proportion of genotypic variance compared to the genotype x environment interaction variance component”. Multiplicative model and biplot invariably grouped similar genotypes (sister lines) in the same cluster and indicated the line CSxV_60 as the “ideal breeding line” in terms of genotypic main effects and stability for yield in
the tested environments. Good stability has been detected for time of anthesis and gluten strength. The observed results are compatible with the hypothesis that the main genotypic effects observed over environments are traceable to the direct and indirect effects of chromatin from *D. villosum*. These lines are ideal starting points for studying individual QTLs with a pre-tested environmental stability and for transferring those QTLs to commercial varieties.
QTL DETECTION IN MAIZE WITH TESTCROSS PROGENIES AS AFFECTED BY RELATED AND UNRELATED TESTERS

FRASCAROLI E.*, CANÈ M. A.*, LANDI P.*, PEA G.**, PÈ M. E.**

*) Department of Agroenvironmental Sciences and Technologies “DiSTA”, University of Bologna, Viale Fanin 44, 40127 Bologna (Italy)
**) Department of Biomolecular Sciences and Biotechnology, University of Milan, Via Celoria 26, 20133 Milano (Italy)

overdominance, QTL effects, RIL population, testcross performance, Zea mays

Populations of Recombinant Inbred Lines (RILs) are valuable materials to carry out genetic analyses, including the detection of quantitative trait loci (QTL), mainly because of their homozygosity and homogeneity. However, for a cross-pollinated species like maize (Zea mays L.), the evaluation of RILs per se can lead to results biased by inbreeding depression. To overcome such a drawback, genetic analyses can be conducted by testing the RILs in hybrid combination, i.e., crossed with testers. The tester choice is, however, a crucial issue, as testers can carry dominant alleles at the QTL for which there is variation, thus hampering their detection and the assessment of their effects. This study was conducted on a maize population of 142 RILs derived from the single cross B73 x H99 in order to (i) evaluate the role played by testers in affecting the QTL detection and (ii) investigate the consistency of QTL effects across testers. RILs were crossed with the related inbreds B73 and H99, and with the unrelated inbred Mo17, thus producing the testcross groups TC(B), TC(H), and TC(M), respectively. The three TC groups (on the whole including 426 TCs) were tested in field trials with two replications in three locations. As expected, the mean value of the TC(M) group was higher than the mean values of the TC(B) and TC(H) for traits related to plant vigor, especially grain yield (GY), plant height (PH), number of kernels per plant (NK), and kernel weight (KW). The heritability values in the three TC groups were quite similar for most traits, whereas were much lower in the TC(M) group for GY and NK. The correlation coefficients for the same trait of TC(B) vs. TC(H), TC(B) vs. TC(M), and TC(H) vs. TC(M) were always positive and often highly significant for traits with high heritability, especially KW and KM (kernel moisture), whereas were much lower or even negative for GY and NK. The number of detected QTL ranged from six for PS (pollen shedding) to 14 for GY and 15 for PH. For traits with high heritability, especially PS, KW, and KM, the most effective tester for QTL detection was the unrelated Mo17; for these traits, there were also several overlaps among the QTL detected in two or all the three TC groups, with the QTL effects being always consistent, i.e., of the same sign. For GY and NK, the unrelated tester Mo17 was the least efficient in allowing QTL detection; moreover, very few overlaps were found between QTL detected in more than one TC group, with the QTL effects being always inconsistent, i.e., of different sign. These findings can be accounted for by considering that GY and NK are controlled by important non-additive effects like overdominance. In conclusion, the three testers proved to play an important role in affecting both the detection of QTL and the estimate of their effects, especially for traits controlled by important non-additive effects.
MOLECULAR MARKERS FOR VE1 AND VE2 VERTICILLIUM RESISTANCE GENES FROM TOMATO ITALIAN GERMPASM AND ITS POSSIBLE USE IN THE SELECTION OF EGGPLANT LINES TOLERANT TO VERTICILLIUM


*) CRA-ISOR, Research Institute for Vegetable Crops Section of Monsampolo del Tronto, Via Salaria 1, I-63030 Monsampolo del Tronto (Italy)
*) CRA-ISOR, Research Institute for Vegetable Crops Section of Montanaso Lombardo, Via Paullese 28, I-26836 Montanaso Lombardo (LO), Italy
***) DI.VA.P.R.A, University of Turin, Via Leonardo da Vinci 44, 10095 Grugliasco (Italy)

Solanum lycopersicum, Solanum melongena, collinearity, breeding, disease

In a preliminary survey on tomato Italian germplasm, an accession derived from the pink ‘Rosa di Sorrento’ local landrace showed resistance to Verticillium wilt (race 1). In tomato, the resistance to race 1 of V. dahliae and V. albo-atrum is conferred by two strictly associated genes, Ve1 and Ve2, which independently confer resistance to the same pathogen.

We developed two new markers for Ve1 and Ve2 on the base of tomato available sequences, based respectively on selective allele-specific PCR amplification and on a PCR amplification followed by enzymatic restriction. These two markers allow the identification of both the allelic forms at the Ve loci and they are of potential interest for the application in marker-assisted selection; furthermore, sequence analysis confirmed that the resistant local “pink” landraces possess the same alleles at the locus Ve1 and Ve2 than that of the most common resistant lines.

Verticillium wilt is considered an important limiting factor in the production of eggplant, especially in Asia and Mediterranean areas. Therefore, the main goals of eggplant breeding are not only the development of high-quality genotypes but also the achievement of Verticillium wilt tolerant hybrids. In spite of the huge variability, a lack of resistance traits in eggplant gene pool is still evident. Thus, the release of eggplant cultivars tolerant to the main diseases and pests has been very limited. Although genetic variability related to resistance traits has been found, many of these genetic sources, often, give contrasting results when employed in breeding programs. Among the wild relatives of eggplant Solanum sodomaeum L. (2n=24) (= S. linneanum) has been considered as possible source of resistance. However, the response of the elite lines derived from the introgression of S. sodomaeum genome in S. melongena via sexual hybridization, followed by backcrosses and phenotypic selection, is quantitative, with different level of tolerance/resistance to Verticillium wilt.

Many earlier comparative genome analysis have been focused on the collinearity of solanaceae genomes, underlining the chance that the similarity of the genomes of the four main Solanaceous crop make feasible the mapping of conserved resistance genes in these related species.
Therefore, the newly developed molecular markers tested in the tomato Italian landraces were tested on *S. melongena* tolerant lines and on its wild relative; the characterisation of the putative eggplant Ve1 and Ve2 homologous is ongoing.
GENETIC DIVERSITY OF ITALIAN LENTIL LANDRACES USING MICROSATELLITE MARKERS

SONNANTE GA., SANTANTONIO M., SONNANTE GI., LIOI L.

Institute of Plant Genetics (IGV)– CNR, Bari

_Lens culinaris, SSR, molecular markers, local varieties_

Lentil cultivation in Italy is mainly based on landraces, local varieties empirically selected by farmers over time and well adapted to the agro-environment in which they have been grown for decades. Most landraces survive on farm, in marginal areas and exposed to a strong risk of genetic erosion and/or extinction. In order to assess genetic diversity and relationships, nine local varieties, two lines and two cultivars of lentil were analysed using microsatellite (SSR) markers. Ten individuals for each landrace and line were used. Plants were grown in a greenhouse and DNA was extracted from young leaves. Sixteen primer pairs, reported to amplify microsatellite regions in lentil, were selected from the published literature. One primer for each pair was fluorescently labelled, so that amplified fragments could be visualized on an automated sequencer. All the used primer pairs produced an amplification product of the expected length. A total of 170 alleles were scored, ranging from 1 to 22 alleles per locus. The diversity parameters were calculated using PopGene software, and resulted to be very low for the selected lines and the cultivars. On the other hand, the lentil landraces analysed in the present study showed quite high values for all the genetic diversity parameters, especially when compared to other legumes cultivated in Italy, such as common bean, or chickpea. These results indicate that the examined landraces retain a high level of genetic diversity among single genotypes of the same landrace. The highest levels of genetic diversity were observed for Castelluccio, Colliano, and Villalba landraces. Microsatellite allele frequencies were used to calculate Nei’s genetic distances, and a UPGMA dendrogram was constructed. As a general rule, lentil landraces were grouped according to their geographical origin. For the ability of SSRs to detect allelic differences between individuals, these sequences have currently become the markers of choice for assessing genetic variation within and among landraces.

This research has been supported by the project PROM, C.I.P.E. (Resolution 17/2003), Italian Ministry of Agriculture (MiPAAF).
IDENTIFICATION OF AFLP FRAGMENTS LINKED TO CREEPING-ROOTEDNESS IN LUCERNE

POLEGRI L.*, ARCIONI S.*, PECETTI L.**, PIANO E.**, PUPILLI F.*

*) Institute of Plant Genetics – CNR, Via della Madonna Alta 130, 06128 Perugia (Italy)
**) CRA – Istituto Sperimentale per le Colture Foraggere, Viale Piacenza 9, 26900 Lodi (Italy)

Medicago sativa, Bulked Segregant Analysis (BSA), creeping rootedness, AFLP, Marker-assisted selection (MAS)

Creeping rootedness (CR) in lucerne is the ability to form adventitious shoots on horizontal roots. CR is an interesting trait to introgress in lucerne because it may confer high tolerance to grazing and better stand persistence. Selection for CR is difficult because the complex genetic control of the trait, which behaves as a QTL with low penetrance: in some individuals the CR phenotype may appear even after 3-4 year of surveys. For these reasons, breeding for CR could take advantage of a Marker-Assisted Selection (MAS) strategy.

To investigate the presence of markers linked to CR in lucerne, AFLP markers were used to screen a segregating population following a Bulk Segregant Analysis (BSA) strategy. This population was obtained by crossing a CR individual (male) showing high expression of the trait with a non-CR individual (female) genetically related to the CR parent. DNA extraction was carried out on 20 F1 hybrids (10 CR and 10 non-CR) selected as the individuals showing the extreme expressions of the trait (estimated on the number of the satellite plants developed by the mother plant over time). This DNA was pooled out to form two CR and two non-CR bulks (composed by 5 individuals each) on wich the BSA strategy was applied, taking the parental DNA as a reference to avoid artifacts.

Seventy $\text{EcoRI-MseI}$ primer combinations were applied on bulk analysis, yielding on average 70 scorable fragments each. Among these, 28% were polymorphic between parents, 45% of which being polymorphic among bulks. In total, 6 amplicons were present in all the CR bulks and absent in non-CR bulks. When the segregation of these amplicons were investigated across the whole mapping population, two of these (M1 and M2) showed non-independent segregation from the CR trait. These two amplicons resulted closely linked each other. The predictivity of the two markers were respectively 80,8% (CR) and 83,3% (non-CR) for M1 and 82,6% (CR) and 80,8% (non-CR) for M2, then showing their potential to be effectively used in MAS for this trait.

Research project funded by Regione dell’Umbria, Programma Interregionale “Sviluppo Rurale” – Sottoprogramma “Innovazione e ricerca-Azioni di innovazione e ricerca a supporto del Piano sementiero”
MORPHOLOGICAL, PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF SANTO STEFANO DI SESSANIO LENTIL

TORRICELLI R.*, SILVERI D. D.**, FERRADINI N.*, VENORA G.***, VERONESI F.*

*) Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali e Zootecniche, Università degli Studi Perugia, Borgo XX Giugno 74, 06121 Perugia (Italy)
**) Agenzia Regionale per i Servizi di Sviluppo Agricolo - Abruzzo, 67039 Sulmona (Italy)
***) Stazione Sperimentale di Granicoltura per la Sicilia, 95041 Caltagirone (Italy)

local variety, Lens culinaris, molecular characterization, AFLP, Image analysis

The Santo Stefano di Sessanio local variety of Lens culinaris Medicus is grown by farmers belonging to a small community of the Abruzzo mountains, at an altitude ranging from 1,000 to 1,600 m a.s.l. Local Italian lentils are prized by consumers for their taste and cooking qualities, a typical example of niche market products able to fetch a relatively high price that makes their cultivation profitable even in marginal areas. The characterisation of S. Stefano di Sessanio local variety is fundamental to make this product identifiable with respect to both worldwide production and similar niche products.

For the above reported reasons, lentil seed samples were collected from local producers while commercial seed sample, purchased on the market, were utilized as controls. A total of 34 entries were compared, 30 local accessions and 4 controls. Morphological and agronomic characterization of lentil accession was conducted in Corfinio (AQ) and Calascio (AQ), respectively, during 2005.

In particular, AFLPs were used to calculate the Dice coefficient of genetic similarity between population pairs. The similarity matrix obtained was then used to produce a UPGMA dendrogram and Principal COORDinates Analysis. Furthermore the seed images acquired by flatbed scanner were elaborated by an image analysis system. Stepwise Linear Discriminant Analysis (LDA) on image analysis parameters were able to discriminate correctly the market lentils and each local varieties.

The results showed the existence of a significant group of local accessions very similar each other in terms of morphological, physiological and molecular traits. It is likely that they constitute the original, still well conserved nucleus of the local variety. Controls have shown different characteristics with respect to S. Stefano di Sessanio accessions. To safeguard and enhance the value of S. Stefano di Sessanio lentil, it is necessary to reach an agreement and to establish a Producer’s Consortium able to become the driving force for a future niche market, avoiding the spreading of alien lens varieties.
A NEW SET OF MICROSATELLITE MARKERS IN ARTICHOKE


*) DiVaPRA, Plant Genetics and Breeding, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (Italy)
**) Plant Research International, Droevendaalsesteeg 1, 6708 PB Wageningen (NL)

*Cynara cardunculus* L., enriched library, microsatellite, linkage analysis

The molecular knowledge of the artichoke (*Cynara cardunculus* var. *scolymus* L., Compositae, 2n=2x=34) genome is still poorly advanced. Till June 2007, just 169 *Cynara* DNA accessions were available on the GenBank database (using the “Taxonomy Browser” tool). Among them, about thirty were microsatellite loci, developed at the Plant Genetics and Breeding laboratories of the University of Torino, using different development approaches. This molecular marker set has to increase in number so as to advance the genetics of the species.

As a matter of fact, microsatellites are useful for management of germplasm, varietal fingerprinting and population genetics studies. Moreover, being codominant markers, they are valuable for map development.

Enriched genomic libraries were built for 10 different repetitive motifs (GA$_{12}$, GT$_{12}$, TCT$_{10}$, TGT$_{10}$, GAG$_{8}$, GTG$_{8}$, TGA$_{9}$, AGT$_{9}$ CGT$_{8}$, GCT$_{8}$, GCC$_{7}$) using a selective hybridisation capture technique, based on oligo-covered nylon membranes.

Eighty-five microsatellite loci were isolated and primer pairs were designed for each locus identified. In order to assess marker diversity and figure out their utility for fingerprinting, PIC (polymorphic information content) values were assessed using 24 *C. cardunculus* samples, including artichoke accessions and individuals from three F$_1$ segregating progenies, obtained by crossing the same clone of ‘Romanesco C3’, as female parent, with either an artichoke (varietal type ‘Spinoso di Palermo’), a cultivated cardoon (var. *altilis*) and a wild cardoon (var. *sylvestris*) genotypes used as pollen sources; the progenies were developed in collaboration with the University of Catania. On the whole, 48 loci showed positive PCR amplification, of which 38 were polymorphic. The estimated PIC values ranged from 0.23 to 0.77, with an average of 0.54.

A linkage analysis of the new microsatellite marker set showing clear segregation pattern in the above mentioned progeny is currently in progress, and they will be valuable as bridging markers to integrate the developing maps.

The establishment of new linkage relationships among such marker loci represents the preliminary step for the identification of chromosomal regions carrying traits of breeding interest, and for their future targeting in marker-assisted breeding programs.
CHARACTERIZATION OF MALE STERILE CLONES OF ARTICHOKE (CYNARA CARDUNCULUS L. VAR. SCOLYMUS)

LO BIANCO C.*, OLMIPERI I.**, MAZZUCATO A.**, CRINO’ P.***, MICOZZI F.*, SACCARDO F.*

*) Dipartimento di Geologia e Ingegneria Meccanica Naturalistica e Idraulica per il Territorio, Università degli Studi della Tuscia Via S. C. De Lellis snr, 01100 Viterbo (Italy)
**) Dipartimento di Agrobiologia e Agrochimica, Università degli Studi della Tuscia, Via S. C. De Lellis snr, 01100 Viterbo (Italy)
***) ENEA C.R. Casaccia, Dipartimento Biotecnologie, Agroindustria e Protezione della Salute, Via Anguillarese 301, 00123 Roma (Italy)

F1 hybrids, globe artichoke, male sterility, reproductive biology

Artichoke (Cynara cardunculus L. var. scolymus) cultivation has a great economic importance worldwide and in particular in Italy with a contribution of about the 40% of the world production. Italy harbors the richest primary gene pool for this crop, maintaining a high number of different ecotypes which are vegetatively propagated. The lack of novel varieties and of suitable propagation techniques, the existence of serious phytosanitary problems and the maintenance of old cropping techniques caused in the last 45 years a continuous decrement of the artichoke cultivation both as cultivated area and as total yield. Traditional propagation methods (eg.: use of offshoots, ovuli or cuttings) entail disadvantages for phytosanitary problems and scarce crop uniformity. In vitro micropropagation may offer several advantages, but implantation costs remain high. Multiplication through seed is a valid alternative to propagate artichoke, because it contributes in optimising cropping techniques, improving the phytosanitary conditions and reducing production costs. As the species is highly proterandrous and allogamous, the material obtained through seed is still today highly heterogeneous and not always adapted to our pedoclimatic conditions.

With the aim of obtaining genetically stable F1 hybrids through the use of male sterile clones, it is crucial the definition of the floral biology of this species. Moreover, it is important to unravel the causes for and the espressivity of male sterility. In this study, the differences in flower development in two male fertile and two male sterile lines have been analysed. The staging of artichoke flowers was based on the length of external flowers in first order inflorescences. With this basis, the size of floral organs and the stage of development have been analysed on a time course spanning the stages from pre-mieiosis to anthesis. The developmental stage in ovules and anthers, relative to the progression of sporogenesis and gametogenesis, has been investigated through staining with aniline blue, acetocarmine and DAPI.
GENETIC VARIABILITY IN A COLLECTION OF WILD CARDOON 
(CYNARA CARDUNCULUS VAR. SYLVESTRIS) BY USE OF 
MICROSATELLITES MARKERS (SSR) 

SABA E.*, RAU D.*, RODRIGUEZ M.**, ATTENE G.* 

*) Dipartimento di Scienze Agronomiche e Genetica Vegetale Agraria, Università degli Studi di Sassari, Via de Nicola, 07100 Sassari (Italy) 
**) Centro per la Conservazione e Valorizzazione della Biodiversità Vegetale, località Surigheddu, Università degli Studi di Sassari 07100 Sassari (Italy) 

Cynara, microsatellites, genetic resources, wild germplasm, population structure 

Wild cardoon (Cynara cardunculus var. sylvestris) is a poliennal species which grows spontaneously in different areas of Sardinia. This species can be used to extract biopharmaceutical products, to produce lignocellulosic biomass and oil from seeds as well as for food preparation. Moreover, it is a potential source of useful genes in breeding programs of cultivated globe artichoke. 

Thirteen populations were collected from seven different ecogeographic areas according to a hierarchical sampling method: three transects were identified per each population and fifteen plants were sampled from each transect. 

A total of 117 individuals (9 per population, 3 per each transect) were analysed with nuSSR and cpSSR molecular markers. For nuSSR, the analysis of the molecular variance (AMOVA) revealed the same level of differentiation both among populations (9.97%) and transects (6.22%). However, a substantial amount of variation was found within population (83.81%). Moreover, molecular analyses revealed a tendency of populations to cluster on the basis of their ecogeographical area of origin. 

The analysis of nuSSR polymorphism has evidenced three distinct genetics groups (not uniformly widespread across Sardinia). In eight out of the 13 populations all of the three genetic groups were present, in 4 populations at least two of the genetic groups were present while only one population was constituted by individuals belonging to one genetic group. CpSSR analysis has evidenced a very low level of variability, with only one polymorphic locus out of the 35 analyzed (2.86%). Two chloroplastic haplotypes have been identified, of which one homogeneously distributed across Sardinia and one localized in the Central-North-West area. A significant association between chloroplastic haplotypes and genetics groups was also observed. 

Field experiments are in progress to obtain a morphological and biochemical characterization of the genotypes belonging to the different genetic groups. These information will likely be useful to identify populations or genotypes interesting for breeding and research purposes.
AFLP GENETIC CHARACTERIZATION IN *ASPARAGUS OFFICINALIS* DOUBLED-HAPLOID (DH) CLONES COLLECTION

RICCARDI P.*, BATTAFAVARANO R.*, CIFARELLI R.**, FALAVIGNA A.***, SUNSERI F.****

*) Dipartimento di Biologia, Difesa e Biotecnologie AgroForestali, Università degli Studi della Basilicata, Via Ateneo Lucano 10, 85100 Potenza  
**) Metapontum Agrobios – SS Jonica 106 km 448,2, 75012 Metaponto (MT)  
***) Ente CRA Istituto Sperimentale Orticoltura, Via Paullese 24, Montanaro Lombardo (LO)  
****) Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università *Mediterranea* di Reggio Calabria, Feo di Vito, 89060 Reggio Calabria

doubled-haploids, genetic characterization, coefficient of similarity, AFLP

Cultivated asparagus (*Asparagus officinalis* L., Liliaceae 2n=20) is a perennial dioecious species, for its tasty stems it is an edible crop of high economic value. Sex is determined by gene(s) on a pair of homomorphic sex chromosomes with homogametic (XX) females and heterogametic (XY) males. However, rarely andromonoecious individuals are observed among the males. These are of XY constitution but able to self-fertilize and produce fertile berries. The F₁ progeny of these selfings segregate in the ratios 1XX: 2XY:1YY, producing the so-called supermales (YY). All-male populations of asparagus crop are widely considered superior to mixed populations, due to higher yield, no contamination of the field by germinating berries and longevity. While they are generally weak and of low productivity themselves, YY-plants are highly valued for the production of hybrid seeds, as all the progeny from crosses with female XX-plants will be of the male XY constitution.

An alternative way to produce supermales is the production of doubled-haploids (DH) *via* anther culture. The regenerated plants should be of haploid constitution but usually double their chromosome number spontaneously, or induced by chemicals, at a very early stage, yielding doubled-haploid XX and YY plants. The *in vitro* anther culture technique has been suggested as the best way to obtain doubled haploid female and male clones of asparagus which can be used as parents of F₁ all-male hybrids. This technique has been applied in Italy during the last twenty-five years and has allowed to release commercially F₁ all-male hybrids.

The present report focused on the genetic characterization of several double haploid accessions, part of an important collection conserved at the C.R.A. Research Institute for Vegetable Crops, section of Montanaso Lombardo. Genetic diversity was analyzed using AFLP molecular markers strategy; forward primers of the nine primer-pairs used were labelled with ABI PRISM fluorescent dye 6-FAM, and the PCR reactions were run on ABI PRISM.

The automated sequence system ABI PRISM combined with fluorescent labelling of expected fragments has been applied as an alternative to radioactivity detection using [³²P] or [³³P]-labelling. This technology provides an automatic and rapid sizing of the fragments through the use of specific internal size standard (GS500-Liz) and allows analysis of fragments. The data collected from each sample were automatically analysed by GeneMapper Analysis Software.
The genetic diversity were estimated; a matrix of presence/absence of DNA fragments has been used for the comparison of the accessions in order to obtain the coefficients of genetic similarity (Dice, 1945). The coefficients have been utilized for the UPGMA (Unweighted Pair Group Method Averages) analysis useful for obtaining a dendrogram among accessions (PAST ver. 1.12 software).

The genetic relationships among double haploid lines together with the morpho-agronomic data should be now utilized in the choice of parental genotypes for F₁ hybrid constitution.
EVALUATION OF GENETIC DIVERSITY IN INODORUS ITALIAN MELON (*CUCUMIS MELO* L.) REVEALED BY ISSR MOLECULAR MARKERS AND PHENOTYPIC TRAITS


*) Research Institute for Vegetable Crops, Section of Ascoli Piceno, Via Salaria 1, 63030 Monsampolo del Tronto (Italy) - nadiaf@insinet.it
**) Department of Life Science, Second University of Naples, Via Vivaldi 43, I-81100 Caserta, (Italy)

*Melon (*Cucumis melo* L.) diploid species (2n=24), is an important horticultural crop in Italy, and is consumed as dessert, vegetable, and ornamental fruit, depending on the area of cultivation and the type of melon. Based on fruit characters such as size, colour and taste, melon is considered the most diversified species in the genus *Cucumis*. The genetic diversity and relationships among 13 *inodorus* Italian melon accessions were assessed by using 100 ISSR primers and 7 morphological traits. The diploaploid line (DH) Nad-1 and the cultivar Charentais T, belonging to the botanical variety *cantalupensis*, were employed as outgroup. The 358 polymorphic bands obtained and the phenotypic traits scored (fruit shape and colour, skin colour and texture, flesh colour, taste and Brix°) were utilized to calculate the genetic distances (GD) by means of Dice and Euclidean indexes, respectively. The UPGMA cluster analyses and the resulting dendrograms were performed on the genetic distance matrices for both molecular and morphological data. The Principal Component Analysis based on the GD matrices was used to show multiple dimensions (2D and 3D) of each group of the accessions in a plot. The correlation coefficient of the GD matrices assessed by the Mantel test was found to be about r= 0.43.

Both phenotypic and molecular analyses allowed us to distinguish the melon genotypes on the basis of both their botanical origin and their morphological characteristics.
THE DEVELOPMENT OF SNPs (SINGLE NUCLEOTIDE POLYMORPHISMS) MARKERS IN FRAGARIA VESCA AND RUBUS IDAEUS AND THEIR TRANSFERABILITITY BETWEEN THE TWO SPECIES

PALMIERI L., SAVIANE S., SORDO M., GRANDO M.S., GIONGO L.
IASMA Research Center, Via E. Mach 1, 38010 San Michele all’Adige (Italy)

Molecular markers are useful for a variety of purposes relevant to crop improvement. The most important of these employments is the indirect assisted selection (MAS). Single Nucleotide polymorphisms (SNPs) are widespread and distributed throughout the genome of most species. Because of their abundance in animal and plant genomes, the relative ease with which they are detected from sequence data and the use in the detection of association between the haplotypes and phenotypes, these polymorphisms could be used as simple genetic markers for population studies and in the indirect MAS exercised during plant breeding.

To date, however, only few SNPs were described in a published work based on quantitative identification of strawberry and raspberry presence, in food products. Identification of SNPs could lead to the development of markers useful for genotyping and for determination of genetic diversity among and between Fragaria and Rubus varieties.

These research works focuses on SNPs discovery on different DNA sequences of Fragaria vesca and Rubus idaeus varieties and on transferability of developed markers between the two genera. Amplifiable DNA was recoverable from strawberry and raspberry leaves and a set of Single Nucleotide Polymorphisms (SNP) markers was started to be developed. To achieve this objective published SCAR primers, developed on Fragaria, were used to amplify and sequencing specific region of strawberry genes. After alignment of different plants enzyme sequences, involved in the specific biochemical pathways, a new set of primers was designed on conserved region. These primers were used to amplify and sequencing raspberry DNA. The polymorphisms found in Fragaria will be investigated in Rubus and vice versa and used for varietal identification. Moreover, technological improvements, make the use of these SNPs attractive for high-throughput screening in the study of genetic diversity, in the marker-assisted selection (MAS) and in food traceability.

ACKNOWLEDGMENTS
This research was financed by InterBerry (Fondo Unico per la Ricerca PAT Trento) and Sicilberry (Regione Siciliana).
MAPPING OF GENES INVOLVED IN PHENYLPROPANOID BIOSYNTHESIS AND QTL ANALYSIS IN C. CARDUNCULUS L.


*) DiVaPRA, Plant Genetics and Breeding, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (Italy) - cinzia.comino@unito.it, ezio.portis@unito.it

**) Dipartimento di Scienze Agronomiche, Agrochimiche e delle Produzioni Animali – sez. Scienze Agronomiche, University of Catania, Via Valdisavoia 5, I-95123 Catania (Italy)

Globe artichoke, cardoon, genetic map, QTLs, hydroxycinnamoyltransferases

The complex *Cynara cardunculus*, includes the globe artichoke (var. *scolymus* L.), the cultivated cardoon (var. *altilis* L.) and the wild cardoon (var. *sylvestris* (Lamk) Fiori). Globe artichoke contributes significantly to the Mediterranean agricultural economy, with an annual production of about 750Mt (more than 60% of global production) from over 80kha of cultivated land; Italy is the leading world producer (FAO data 2006: http://faostat.fao.org/). A better knowledge of artichoke and cardoon genetics will be essential to move to a crossing strategy for breeding. In particular, it will be advantageous the establishment of frameworks of linkage relationships to allow the identification and localization of genes controlling important yield traits. *C. cardunculus* is also a source of biopharmaceuticals and its leaf extracts have been widely used in herbal medicine as hepatoprotectors and choleretics since ancient times. The chemical components of the leaves have been found rich in compounds originating from the metabolism of phenylpropanoids and the major species present are the di-caffeoylquinic acids (e.g. cynarin), and their precursor CGA, a soluble phenolic which is widespread throughout the plant kingdom.

We generated the first genetic maps of globe artichoke by analysing an F1 population created by crossing a clone of ‘Romanesco C3’ (a late-maturing, non-spiny type) with ‘Spinoso di Palermo’ (an early-maturing spiny type), using AFLP, M-AFLP, SSR and retrotrasposon based SSAP markers. Moreover we identified and characterized two acyltransferases: HCT (hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyltransferase) and HQT (hydroxycinnamoyl-CoA quinate: hydroxycinnamoyltransferase) which are involved in the synthesis of caffeoylquinic acids, substrates of compounds such as cynarin.

Here we report on SNP analyses in HCT and HQT sequences of the two mapping parents to place the genes on the globe artichoke genetic linkage map. Furthermore, we report on the development of new genetic maps based on F1 progenies obtained by crossing the same clone of ‘Romanesco C3’, previously used as female parent, with either a cultivated cardoon (‘Altilis 41’) and a wild cardoon (‘Creta 4’) genotypes used as pollen sources. Wide cross populations of this type are suitable for investigating the genetic control of quantitative characters in exotic genetic backgrounds; both wild and cultivated cardoon represents the most straightforward resource to exploit for globe artichoke improvement, since they are full cross-compatible to it. Since *C. cardunculus* is easily vegetatively propagated, the mapping populations are immortalised, and thus were grown in contrasting environments to investigate genotype x environment interaction for important commercial traits.
ISSR ANALYSIS OF SEEDLINGS FROM CRYOPRESERVED SEEDS OF AN ANCIENT CITRUS COLLECTION IN FLORENCE

DE CARLO A.*, BENELLI C.**, LAMBARDI M.**

*) IGV/Istituto di Genetica Vegetale, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (Italy)
**) IVALSA/Istituto per la Valorizzazione del Legno e delle Specie Arboree, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (Italy)

Citrus, cryopreservation, ISSR, zygotic and nucellar embryos

In recent years, many studies have dealt with the cryopreservation of the genus *Citrus*. And, as a result, efficient procedures of vitrification or dehydration have been reported for various organs and tissues, such as shoot tips, seeds, embryonic axes, somatic embryos, ovules and embryogenic callus. Among them, the cryostorage of embryonic axes and entire seeds from polyembryonic species allows the preservation of nucellar embryos, which are genetically identical to the maternal parent. Hence, the polyembrionic seeds can be considered a valuable material for the long-term preservation of citrus germplasm. This study explored the possibility of using the cryogenic technology for the preservation of a Medicean *Citrus* collection, maintained at the botanical garden of the “Villa Reale di Castello” in Florence, Italy. The *Citrus* collection was initiated by Cosimo I de’ Medici in the XVI° century, and it includes more than 600 accessions, preserved mainly in big earthenware basins. An effective procedure of seed dehydration and direct immersion in liquid nitrogen (“one-step freezing”) was initially developed for a sample of five polyembryonic *Citrus* accessions, i.e., *C. aurantium* ‘Foetifera’, *C. volkameriana*, *C. lumia* ‘Pyriformis’, *C. sinensis* and the hybrid *C. aurantium* x *C. paradisi*. The initial moisture content of seeds was between 33% and 55%. Seed dehydration was obtained in open Petri dishes, exposed to sterile air flow. All the accessions showed adaptability to seed cryopreservation, after the seeds were appropriately dehydrated between 25% (*C. lumia*) to 15% (*C. aurantium* x *C. paradisi*). Maximum germinability ranged from 27% (*C. aurantium*) to 100% (*C. aurantium* x *C. paradisi*). With the only exception of *C. volkameriana*, only one seedling per seed was obtained after the dehydration/cryopreservation procedure. Afterwards, in order to validate the procedure for the preservation of this ancient germplasm, the ISSR (Inter-Simple Sequence Repeat) analysis was carried out to ascertain the sexual or apomitic origin of seedlings from cryopreserved seeds. The DNA was extracted from the mother plants and from the seedlings which developed after the seeds underwent to the dehydration/one-step freezing procedure. For ISSR analysis, four primers anchored at the 3’ end by 2 arbitrary and degenerate nucleotides were selected: (AG)$_8$YC, (AG)$_8$YG, (GA)$_8$YG (according to Fang and Roose, 1997. TAG 95:408-417), and (AC)$_8$YG (according to Sankar and Moore, 2001. TAG 102:206-214). The amplified fragment sizes were from 200 to 1300 bp. The number of fragments per primer ranged from 5 (with (AG)$_8$YC) to 12 (with (AC)$_8$YG). For the accessions from which only one seedling per cryopreserved seed was obtained, the molecular analysis confirmed their nucellar origin. As regards *C. volkameriana*, the molecular analysis showed the occasional development of also the zygotic embryos, although at a very low ratio in comparison with the nucellar embryos. With *C. volkameriana*, as well as with other polyembrionic *Citrus*
species which could have the same behaviour, this fact should be considered when using the
cryopreservation technique for the long-term preservation of clonal germplasm.
IDENTIFICATION OF UNREDUCED GAMETE ORIGIN IN CITRUS INTERPLOID CROSSES (2X X 4X) BY MEANS OF SSR MARKERS


*) Dipartimento S.En.Fi.Mi.Zo. - Sez. Patologia Vegetale e Microbiologia Agraria, Università degli Studi di Palermo
**) Istituto di Genetica Vegetale, CNR, Corso Calatafimi 414, 90129 Palermo
***) ENEA C.R. Casaccia Plant Genetics and Genomics Section (026), S.M. di Galeria (Roma)
****) Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università degli Studi del Molise, Via De Sanctis, 86100 Campobasso

flow cytometry, somatic hybrids, diploginy, capillary electrophoresis

One of the most important goal in Citrus genetic improvement is the obtainment of seedless cultivars. Among the available strategies, we used the accomplishment of suitable interploid crosses (2X x 4X). To this purpose, three allotetraploid somatic hybrids have been used as pollen parent in sexual crosses with diploid ‘Femminello’ lemon, and autotetraploid ‘Dancy’ mandarin as pollen parent in crosses with diploid grapefruit and mandarin. From flow cytometry, among the 158 analyzed genotypes, 14 tetraploid genotypes were detected. We tested SSR analysis coupled with capillary electrophoresis fluorescence-based technology for the analysis of tetraploid genotypes; all the tetraploid plantlets showed genetic segregation compared to parent genotypes, confirming their zygotic origin. To understand the opportunity to use these tetraploid hybrids for further genetic studies, it has been necessary to identify the cytological mechanisms underlying the ploidy level (4x) in the obtained progeny. A better understanding of micro and macrosporogenesis process is important to clarify the ploidy level in the progenies. By the whole of the analyzed tetraploid hybrids, we found that diploginy (diploid ovule development) has occurred. Therefore, the analyzed tetraploid hybrids with their improved genetic female background (diploid ovule) can be considered very useful in our citrus genetic development program; moreover, the obtainment of tetraploid hybrids allow a greater number of back crosses useful to eliminate negative traits.
SNPs IDENTIFICATION FOR DISCRIMINATION OF OLEA EUROPAEA CULTIVARS

SCIALPI A., BOGANI P., MADIAI L., BUIATTI M.

Department of Animal Biology and Genetics, University of Florence, Via Romana 19, 50125 Florence (Italy)

Olea europaea, SNPs, chs genes

Olive trees cultivation plays a major part in the Mediterranean agricultural tradition. Large is the number of cultivars grown in this area and the identity of most of them is closely linked to specific regions. Molecular tools able to discriminate different cultivars and to assign individuals to varietal populations have become an important issue for the safeguard of local genetic resources.

A wide range of molecular markers have been used for cultivars discrimination in olive, but not many SNPs markers have been identified until now. These markers are very useful since they are genetically stable and can be associated with phenotypic traits.

The objective of this work is to explore the possibility to identify SNPs, able to discriminate among olive cultivars, within genes that encode the enzyme chalcone synthase (CHS), that catalyzes the first step of the biosynthetic pathway of anthocyanin as well as of other flavonoid compounds.

With this aim exon I, intron and a part of exon II of two different chs genes were amplified in ten olive cultivars, typical of different regions of the Mediterranean basin, and in the feral form O. europaea var. sylvestris. SNPs were identified within the coding sequences and these changes were mostly silent or resulting in like-for-like amino acid changes. SNPs and INDELs were found within the introns. The number of transitions was larger than for transversions among the single base changes identified. Overall analysis of the obtained results suggested that the polymorphic patterns in the amplified regions can be able to characterize different cultivars and that the polymorphisms set allows clustering the varieties into different groups.
TRANSFERABILITY OF SIX 48-PLEX SNPSET THROUGH SNPlex™ GENOTYPING SYSTEM WITHIN THE GENUS VITIS


*) Department of Genetics and Molecular Biology, IASMA research centre, Via Mach 1, I-38010 San Michele a/Adige (Italy)
**) Department of Viticulture and Enology, University of California, One Shields Avenue, CA-95616 Davis (USA)
***) UMR 1097 Diversité et adaptation des plantes cultivées, INRA-SupAgro - IRD, 2 place P. Viala F-34060, Montpellier (France)

# these two authors equally contributed to the present work

SNPs, SNPlex™, Vitis spp., genetic diversity studies

Due to their abundance in plant and animal genomes and their suitability for automation, Single Nucleotide Polymorphisms (SNPs) are being considered attractive markers for high-throughput use in marker-assisted breeding, expressed sequence tag (EST) mapping, integration of genetic and physical maps and association studies. Several SNP identification methods are available such as re-sequencing of PCR amplicons, electronic SNP (eSNP) discovery in EST and shotgun genomic libraries. To date many different strategies have been developed for high-throughput detection of SNPs, including the recent SNPlex™ Genotyping System. Based on the polymorphisms discovered by sequencing the genome and by assembling the two haplotypes of *Vitis vinifera* L. cv Pinot Noir, in the present work we have tested the transferability of 288 candidate eSNPs (six 48-plex SNPset) among 71 genotypes within the genus *Vitis*. Each locus has been studied in 37 North American accessions (referring to 26 *Vitis* species), 10 *V. vinifera* ssp. *sylvestris* accessions and 24 *V. vinifera* ssp. *sativa* varieties. In particular, within *V.v. sativa*, genotypes with different parentage degree from Pinot Noir have been considered. Here we report assay pass rates, call rates, call confirmation by resequencing, inter-specific SNP transferability efficiency and SNP-based genetic diversity studies.
GENERATION, ANNOTATION AND ANALYSIS OF ESTs FROM PINUS PINEA MILL.


*) Scuola Superiore Sant’Anna, International Programme in Agrobiodiversity, Via Anguillarese, c/o Enea Casaccia, 00060 Santa Maria di Galeria (Roma) 
**) Dipartimento di Biotecnologie Agraria, Genexpress, Università degli Studi di Firenze, Via della Lastruccia 14, 50019 Sesto Fiorentino (Italy) 
***) Istituto di Genetica Vegetale, Sezione di Firenze, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, 50019 Sesto Fiorentino (Italy) 
****) Dipartimento di Agrobiologia e Agrochimica, Università della Tuscia, Via Camillo de Lellis, 01100 Viterbo (Italy) 
*****) Dipartimento di Biotecnologie Agraria, Sezione di Genetica, Università degli Studi di Firenze, Via Maragliano, 50134 Firenze (Italy)

ESTs, EST-SSRs, genetic diversity, Pinus pinea

Stone pine (*Pinus pinea* L.) has been used since ancient times for timber, landscape architecture and for its large edible seeds. Consequently, it was extensively planted around the Mediterranean by Etruscans, Greeks, Romans and Arabs and its natural range is now difficult to circumscribe.

Recent results showed evidence for an almost complete lack of cpSSR variation across the full species range, a feature never encountered so far in any other widespread plant species. These results are consistent with an earlier report based on allozyme markers that detected only one polymorphic locus. Stone pine appears to have passed through a severe and prolonged demographic bottleneck.

To date, the molecular marker of choice for population genetics studies of tree species have been simple sequence repeats (SSRs). Expressed sequence tags (ESTs) are sequenced portions of messenger RNA and offer an alternative source for marker discovery. EST markers can be easily assayed by the polymerase chain reaction (PCR) and are known to be more conserved and thus more easily transferable between species than microsatellites or anonymous markers. Sequencing cDNA libraries is a cost-effective way to target genes and avoid repetitive DNA and offers an alternative route for SSR marker discovery, particularly for the repetitive genomes found in conifers.

Here we show the first results of the sequencing of a cDNA library of *P. pinea*. In this survey we sequenced a total of 1920 ESTs. A total of 1680 unique sequences were identified, of which 75% had sequence similarity with GeneBank entries, using BLASTX algorithm. Twelve EST-SSR were identified and used to screen variation within and among populations. Causes for the low genetic diversity but broad geographic distribution of *P. pinea* are discussed.
CHENOPODIUM QUINOA: A NOVEL CROPS FOR OUR FIELDS? FIRST
STEP: GERMPLASM EVALUATION

TAVIANI P., MENCONI L., PIERONI G., RUBINI A., DAMIANI F.

Institute of Plant Genetics – CNR, Research Division Perugia, Via Madonna Alta 130, Perugia

novel crops (exotic species), quinoa, genetic diversity, functional foods

Introduction of exotic crops or recovery and valorisation of germplasm resources are two strategies to diversificate farmers’ production. In the last years, while the second approach has been widely persecuted, the first has been poorly exploited. Current interest for functional foods and a new market demand, the necessity to explore novel potentialities for our agriculture, also at the light of global climatic changes, urge to identify new crops from other environments suitable for our agricultural system.

Quinoa (*Chenopodium quinoa* Willd subsp. *quinoa*, 2n =4x=36) is an annual plant originated in South America and cultivated in the Andes. It is a pseudocereal featured by a high quality and high level of protein, absence of gluten, good resistance to abiotic stresses. Preliminary experiments carried out in Europe proved its suitability to be cultivated *ex-situ* but, to offer marketable product, agronomic and breeding researches are necessary.

Twelve accessions of quinoa have been collected from scientific institutions, seed companies and local markets. In a spaced plant trial they were analysed for morphological, phenological traits (IPGRI descriptors) and seed production. Molecular diversity accession was tested using the SSR (Simple Sequence Repeat) technique, also with the aim to select accession-specific markers suitable to study the mating system of the species. At the same time three accessions were utilized to investigate the optimal seeding period as well as to analyse, in controlled environment, phenological phases at different photoperiodic regimes.

Very preliminary observations showed a large variability among and within accessions, so it is easier to select lines suitable for Italian environments as well as for planning breeding experiments. The identification of the optimal combination accession x cultural practices (seeding time, weed control, length of the growing period) is fundamental to introduce such crop in Italy.
PHYTATE AND MICROELEMENTS CONCENTRATION IN A COLLECTION OF DURUM WHEAT CULTIVARS

FICCO D.B.M., RIEFOLO C., NICASTRO G., DE SIMONE V., CATTIVELLI L., DE VITA P.

C.R.A. Istituto Sperimentale per la Cerealicoltura, S.S. 16 km 675, 71100 Foggia (Italy) – pasquale.devita@entecra.it

phytate, phosphorous, microelements, durum wheat

Most of the inorganic phosphorus (Pᵢ) present in mature cereal seeds (between 40 to 80%) is stored as phytate, an anti-nutritional factor, that forms complexes with minerals such as Ca, Mg, Zn and Fe, and reduces the total P bioavailability. The present study was undertaken to determine the variation in Pᵢ and mineral concentration in the whole grains in 93 durum wheat (Triticum durum Desf.) cultivars representative of old and modern germplasm adapted to the Mediterranean conditions and to identify nutritionally superior durum wheat cultivars that possess low phytate content and high concentration of mineral elements in their whole-wheat flour. These cultivars were grown under the same field conditions during 2004-2005 at Foggia, Italy and during 2005-2006 at Foggia and Fiorenzuola d’Arda - PC, Italy. The phytate of each durum wheat cultivar was estimated indirectly by using the microtitre plate assay evaluating the Pᵢ absorbance at 820 nm, while the Cu, Fe, Mn, Ca, K, Mg, Na and Zn mineral contents were determined by ICP/OES. The results obtained showed a large genotypic variation of all micronutrients evaluated. In particular, the contents of Zn and Fe among the 93 durum wheat cultivars ranged from 23.2 to 58.5 ppm for Zn with an average of 34.0 ppm and from 26.2 to 97.3 ppm for Fe with an average of 43.4 ppm. Regarding the Pᵢ grain content the mean values recorded across the years and the locations ranged from 0.32 mg g⁻¹ to 1.09 mg g⁻¹ showing a positive correlation to all minerals with the exception of Cu and Zn suggesting the possibility to design a specific breeding program for improving the nutritional value of durum wheat cultivars through the identification of parental with low-Pᵢ and high minerals concentration in whole grains.
PHENOTYPING AND CATALOGUING OF HYSTORICAL ITALIAN RICE GERMPLASM

CAVIGIOLO S.*, GREPPI D.*, VALLINO M.*, LANZANOVA C.*, TAMBORINI L.**, LUPOTTO E.*

*) C.R.A- Istituto Sperimentale per la Cerealicoltura – Sezione specializzata per la Risicoltura - Vercelli (Italy)
**) ENSE - Milano (Italy)

Rice (Oryza sativa L.) is one of the most important cereal crop, and the staple food of more than half the world’s population. Rice genetic resources, represented by traditional and modern varieties, genetic stocks and breeding lines, are the basis of world food security. Characterisation, conservation and rejuvenation of these genetic resources are important to limit genetic erosion and to help in developing new varieties in different breeding programmes.

A field experiment was conducted in order to study the diversity in morphological, phenological and quality traits of hystorical Italian rice germplasm and to investigate the possible relationships between these traits. The experiment were carried out at C.R.A-Istituto Sperimentale per la Cerealicoltura at Vercelli, during 2006. A total of 112 rice varieties were grown: seventy-one of these varieties represent the hystorical rice germplasm released in Italy between 1850 and 1940, and 41 varieties represent the modern rice varieties, released in the last 10 years. Rice varieties were sowed in single plots 4-5 m long, in 6 rows (two rows for each variety). Rice was drill seeded at approximately 5 g of seed per row, in dry conditions. Permanent flooding was established at 3-4° leaves development stage and the soil was then kept submerged until 1 month before harvest.

During the cropping season, the main morphological and phenological traits were evaluated following the “Standard Evaluation System for Rice, IRRI 2002“ (www.irri.org) and the “Guidelines for the conduct of tests for distinctness, uniformity and stability, 2004” (www.upov.int).

At maturity, rice samples were collected and evaluated for some important grain quality traits, such as amylose and protein content, milling yield and processing quality (amylographic profile using Rapid Visco Analyzer-Foss).

Results showed there was considerable genetic variation between groups (hystorical and modern) and among rice varieties for each group, for all traits examined. Rice varieties varied greatly in term of total plant height from the old varieties, in which plant height ranged from 74,5 to 137,2 cm, being 72% of these higher than 100 cm. The group including modern varieties, clearly showed the effect of the exploitation of sd-1 gene (semidwarf) and plant height ranged from 67,0 to 110,0 cm. Most of these varieties (84%) were shorter than 100 cm. Difference among varieties were also observed in terms of: growth cycle (sowing-maturity interval), panicle length, panicle type and plant architecture. There was large variation among genotype for all traits concerned with grain size and shape. Brown rice grain length ranged from 4,29 to 6,97 mm into hystorical rice group and 5,01 to 8,05 mm for modern rice group. Grain length – to – width ratio ranged from 1,43 to 2,66 mm, and from 1,65 to 3,39 for the old varieties and modern varieties respectively. Grain length was
negatively correlated with grain width but positively correlated with length–to–width ratio. In year 2007 another set of about the same number of genotypes will be analyzed thus completing the survey which will result in a complete data base that will be available on line.

The study is performed within the framework of the project Risorse Genetiche Vegetali financially supported by Italian MIPAAF (D.M.18862/06).
The preservation of plant biodiversity has become a strategic point for scientific research, due to the continuous loss of genetic variability in the germplasms of the main crops. Breeders and researchers have become more aware of the need of maintaining genetic diversity among hybrid varieties and improving the management of genetic resources. However, a germplasm collection as a source of genetic diversity must be well characterised for efficient management and effective exploitation. The analysis of the amount and distribution of genetic variation within and among population of a species can in fact provide basic information for breeding programs and for the establishment of projects to conserve genetic diversity.

The Italian maize germplasm is one of the most interesting in Europe for its large genetic variability and richness of local ecotypes. The maize collection stored at the Maize Section of CRA – ISCE, composed of more than 4300 accessions of different origin, is unique in Italy; particularly relevant is the presence of 620 traditional Italian populations, collected before the introduction of hybrids.

Several programs have been developed in the past few years to preserve this germplasm and valorise its potential as a source of genetic variation. The researches addressed several topics: a) maintenance of the collection through the regeneration of the genotypes in the field and their morphological description; b) evaluation of hybrids yield and stability in Italian environments; c) characterisation of special traits of the grain (waxy, ae, flint) for industrial uses; d) analysis of the grain components (protein, lipid, starch, fibre, carotenoids) and evaluation of the nutritional quality, both as food and feed; e) identification of genes or alleles with beneficial effects for the plant, and analysis of their expression in the tissues; f) evaluation of the resistance to fungal pathogens, using both in vivo and in vitro tests; g) valorisation of the endemic genotypes for the production of typical products, also preserving their traditional uses.

The current research activities are focused on the organization of a data base which could serve as a reference for the best exploitation of the genetic diversity present in the germplasm.
GENETIC DIVERSITY OF 54 MAIZE LANDRACES

HARTINGS H., BERARDO N., LAZZARONI N., PIRONA R., MOTTO M.

CRA – Istituto Sperimentale per la Cerealicoltura – Bergamo, Via Stezzano 24, 24100 Bergamo

Zea mays L., germplasm diversity, morphological traits, molecular markers, Lombardy

Fifty-four Italian maize landraces cultivated up to the 1950s in Lombardy were considered in this study. All entries were grown in a randomized complete block design with three replicates. Morphological traits were measured from 10 competitive plants. The population displayed ample variation for earliness, plant architecture traits, tassel traits, and ear and kernel characteristics. Relationships between traits were investigated using correlation coefficient estimations and principal component analysis. The first four components (PC) accounted for 59.7% of total variance. Cluster analysis was used to reveal the association between the accessions. Genetic similarity was calculated from the morphologic data as Jacard’s similarity coefficients and used to perform UPGMA cluster analysis. The similarity coefficients ranged from 0.07 to 0.28. Cluster analysis placed the 54 accessions into distinctive groups. The first main cluster included most of the accessions with small kernels, a high number of rows and short vegetative cycle. Within the second cluster two distinctive sub-clusters were clearly identified. A first sub-cluster is based on the Nostrano type and derived forms, while in the second sub-cluster we find most of the varieties from Valtellina, an alpine valley. In the third main cluster we find mainly semi-early, eight-row and derived types. The fourth main cluster is also heterogeneous and consists of several semi-early types and of dominants of Marano.

Molecular genotyping was carried out using AFLP with ten primer pairs producing 284 polymorphic AFLP bands from the 54 varieties analyzed. Scoring of the markers allowed the construction of a binary array, which was consequently utilized to compute GS and genetic distance (GD = 1- GS) values for all pairs of accessions studied. GD value ranged from 0.124 to 0.62 and averaged on 0.437 ± 0.012 for the entire data set. Distance measures were subsequently used to construct a hierarchical tree using the UPGMA method. Cluster analysis based on AFLP markers resulted in a clearer separation of each accession in comparison to morphological data. In the dendrogram generated from the data set, the different populations were divided into four major clusters. The first main cluster is heterogeneous and includes several varieties of Nostrano and Rostrato derivatives. The second main cluster mainly consisted of varieties with a short vegetative cycle (quarantini and cinquantini). The third main cluster is highly heterogeneous and contains several varieties of early and semi early eight-row derivatives. This major cluster was further divided in two sub-clusters: one containing representatives of the populations with long cylindrical (flint) ears of the Nostrano dell’Isola group and the second containing types from Valtellina. The forth main cluster is highly differentiated and contains mainly early type accessions that may be seen as a case of particular adaptation.

Information for an appropriate conservation and management of maize germplasm is reported and discussed.
Common bean (*Phaseolus vulgaris* L.) is the world’s most important grain legume for direct food consumption, especially in Latin America and Africa. Although little is known about its genomic organization, the evolution and domestication history of this species that has been intensively studied over the last few years.

*P. vulgaris* is thought to have originated in a region encompassing Ecuador and northern Peru, and dispersed both northwards and southwards establishing the Mesoamerican and Andean gene pools, respectively. Independent domestication took place in these two gene pool.

The main objectives of this study were to (1) investigate genetic diversity within and among populations of common bean, (2) examine the population structure, and (3) determine the extent and genomic distribution of Linkage disequilibrium (LD) using AFLP markers.

We have analyzed the DNA of 199 genotypes of *Phaseolus* from the three known gene pool (Andean, Mesoamerican and Ancestral) with 418 AFLPs fragments resulting from 19 primer combinations.

Population structure was investigated using different model-based inference framework. Overall, a clear separation was found between Andean and Mesoamerican gene pools. Moreover the results showed a very high level of LD in domesticated compared to the wild forms in both Andean and Mesoamerican gene pools.
The main aim of this study was to use an AFLP-based, large-scale screening of the whole genome of *Phaseolus vulgaris* L. to determine the effects of selection on the structure of the genetic diversity in wild and domesticated populations.

We first used pooled DNA samples, seven each of wild and domesticated populations of *P. vulgaris* were studied using 2,506 AFLP markers (on average, one every 250 kb). About 10% of the markers were also analysed on individual genotypes and were used to empirically infer allelic frequencies from bulk data. In both datasets, we tested the departure from neutral expectation for each marker using an $F_{ST}$-based method.

Moreover, we tested with 19 AFLP primer combination a large set of accession from the three known gene pool of *P. vulgaris* (Andean, Mesoamerican and ancestral) in order to highlight the signature of selection under domestication within and between gene pools.

The most important outcome is that a large fraction of the genome of the common bean appears to have been subjected to effects of selection during domestication. We also mapped and classified the markers obtained in individual genotypes according to their proximities to known genes and QTLs of the domestication syndrome. Most of the markers that were found to be potentially under the effects of selection were located in the proximity of previously mapped genes and QTLs related to the domestication syndrome.

Overall, our results indicate that domestication appears to have affected not only target genes, but also a large portion of the genome around these genes. These “domestication islands” have probably experienced a higher level of isolation between the wild and the domesticated forms in comparison with the rest of the genome probably because of linkage to the loci selected during domestication.

Thus, the regions of the genome surrounding the major domestication genes are particularly interesting to tag the introgression from wild relatives into modern cultivars.

As most of the markers that are under the effects of selection are linked to known loci related to the domestication syndrome, we conclude that population genomics approaches are efficient in
detecting QTLs. We also present a method based on bulk DNA samples that is effective in pre-screening for a large number of markers to determine selection signatures.
DOMESTICATION HISTORY IN COMMON BEAN (Phaseolus vulgaris L.): INFERENCES FROM MULTILOCUS SEQUENCE DATA


*) Department of Plant Science, University of North Dakota, Fargo, North Dakota 58105 (USA)
**) Dipartimento di Scienze degli Alimenti, Facoltà di Agraria, Università Politecnica delle Marche, Via Brecce Bianche, I-60131 Ancona (Italy)
***) Genomics and Bioinformatics Program, North Dakota State University, Fargo, ND, 58105 USA

Phaseolus vulgaris L., domestication bottleneck, coalescence simulations, sequence polymorphism

DNA sequence polymorphism carries genealogical information and allows for testing hypotheses on selection and population history, especially through coalescent-based analysis. Understanding the evolutionary forces at work in plant domestication and subsequent selection is of critical importance for the management of genetic resources.

In this study, we surveyed DNA sequence diversity in the wild–domesticated complex of common bean (Phaseolus vulgaris L.). Cultivated common bean has two distinct gene pools (Middle American and Andean). Little is known about the effect of the separation of the common bean into gene pools and the subsequent domestication process on genetic diversity at the DNA sequence level.

Our objective is to study the effects of domestication and improvement on loci near and far from mapped domestication loci by sequencing 3’ portions of different genes in the three groups (wild, landraces and cultivar).

Overall, we analysed a sample of genotypes chosen on the basis of SSR data in order to represent the largest diversity within each set of accessions. We performed several statistical tests to identify the signature of selection due to domestication and crop improvement.

This very promising research provides the tools to identify genes of potential agronomic importance and to determine the effect of domestication and breeding on the structure of genetic diversity in the common bean genome.
Germination potential of *V. corymbosum* and hybrids of 23 genotypes was tested under seeds pre-treatment for dormancy breaking. The plant genetic material included ‘Blue Crop’, ‘Brigitta’, ‘Chandler’, ‘Elliott’, ‘Jubilee’, ‘Legacy’, ‘Ozarkblue’, ‘Rubel’; ‘Fol35’, ‘Misty’, ‘Nui’, ‘Reka’, ‘RH38’, ‘RH48’, ‘RH52’, ‘Star’. After extraction by maceration, seeds were manually classified under stereoscopic microscope. The applied tests were focused on breaking dormancy mechanisms using different seed pre-treatments: (A) fresh seeds were seeded as soon as extracted from fruit; (CH2) were cold stratified at 2°C for 12, 33 and 90 days; (CH3) cold stratified for 12, 33 and 90 days and imbibed in gibberillic acid previous to seedling; (CH4) cold stratified for 12, 33 and 90 days and scarified with sulphuric acid previous to seedling. Furthermore, a test was added on fresh seeds extracted from 100 days stratified fruits and treated with GA3 for two clones of ‘Blue Crop’, ‘Chandler’ and the hybrid ‘Fol 35’.

Number of seeds for each genotype was variable in relation to availability.

The experiment has been carried up closely to nursery environmental conditions. Winter protection was provided, together with natural illumination, and temperature of 18-20°C. Individual seedlings per genotype and treatment were seeded on a substrate composed of (1.1:1) perlite, vermiculite and sand. Irrigation was manually with spry applicator and acidified water (pH 4-5,5). Records were registered weekly.

Observed germination rates compared to non germinated, analysed with $\chi^2$ test, indicate significant differences ($P=0,001$) between different genotypes out of treatment effects. Total germination performance varies from 4 to 100%, on an average of 31%.

‘RH38’ germinated up to 100%, ‘Chandler A’ up to 100% ‘Legacy’ up to 94%, ‘RH48’ up to 84%, ‘Blue Crop A’ up to 92 %, ‘Chandler B’ up to 89%, ‘Ozarkblue’ up to 86%, ‘Blue Crop B’ up to 82 %, ‘Fol 35’ up to 66 %, ‘Duke’ up to 62%.

Positive effects were observed with gibberillic acid imbibition, cold stratification for 33 days or interaction between both factors.

The germination percentage increased with stratification and the results strengthened by hormones addition. 90 days of stratification tend to reduce germination values compared to 33 days.

For the pre-treated seeds with stratification with 33 days and GA3 addition the phase of major rates of the germination performance lasted around 3-4 weeks, compared with A test (5 weeks) so that is significant the effect on acceleration and homogeneity that seem to overcome light requirements. Sulphuric acid treatments showed low and uneven results. When seeds were extracted from 100 days stratified fruits, treated with GA3 and freshly seeded, results did not improve.
The main aim of this study is to compare the population genetic structure at cpDNA of *Betula pendula* and *Betula pubescens* and draw phylogeographic inferences. We used both cpDNA PCR-RFLP and microsatellites to genotype *B. pendula*, *B. pubescens* and to a limited extent, *B. nana*, in 55 populations across Eurasia. A spatial AMOVA (SAMOVA) was used to identify major clusters within each species. The low level of phylogeographic structure previously observed in *B. pendula* was confirmed and the SAMOVA analysis only retrieved two major clusters. In contrast, seven clusters were observed in *B. pubescens*, although the overall level of population differentiation was similar to that of *B. pendula*. We detected a difference in population genetic structure between the two species despite extensive haplotype sharing. It is difficult to ascribe this difference to a single factor, but differences in ecology between the two species may provide part of the explanation. For both species, the contribution of southern Western populations to the recolonization after the Last Glacial Maximum seem to have been limited and eastern and western European populations apparently had different histories.
GENETIC STRUCTURE OF *CUPRESSUS SEMPERVIRENS* L. (CUPRESSACEAE) IN ITS PRIMARY AND SECONDARY DISTRIBUTION AREA ANALYSED THROUGH NUCLEAR MICROSATellites


*) Consiglio Nazionale delle Ricerche, Istituto per la Protezione delle Piante, Sesto Fiorentino  
**) Consiglio Nazionale delle Ricerche, Istituto di Genetica Vegetale, Sesto Fiorentino  
***) Dipartimento di Biotecnologie Agrarie, Università degli Studi di Firenze, Firenze

The origin of cypress has to be identified in the Greek islands (Crete, Samos, Rodos, Kos, and Symi), in Cyprus, Anatolia, Syria, Lebanon, and Iran; in these regions cypress occurs mostly as disjunct populations. The present distribution of this species comprises most of the Mediterranean region and results far broader than it was originally; its presence outside of the original area dates back to the ancient Mediterranean civilisations. The genus *Cupressus* L. comprises 16-24 species, depending on taxonomic interpretation, twelve of them from the Old World. Three species are autochthonous in the Mediterranean region, where cypresses have been cultivated during the last thousands of years as ornamental and for several purposes, including quality of wood, and drought tolerance, which makes cypresses largely utilised against soil erosion. We investigated the genetic structure of *C. sempervirens* in its primary and secondary distribution area with molecular markers (nuclear microsatellites). We collected nineteen natural populations from Turkey, Greece, Syria, and Jordan, and we compared them with twelve Italian stands. The genetic structure was analysed through the utilisation of six nuclear microsatellites. The main goals of our investigation were: i) to quantify the genetic diversity in cypress natural populations and; ii) to infer the possible origin of Italian naturalised populations. Results showed that: significant departures from panmixia were observed for some loci–population combinations. A strong genetic differentiation among populations (*G_{ST}= 0.15*) was found, and a significant correlation between genetic and geographic distance was observed. A high proportion of the total variance is due to differences between the two groups of populations (Italy and the others). Discussion about the possible origin of the Italian populations as well as about the possible factors originating the deficit of heterozygosity in this species is reported.
THE DISTRIBUTION OF QUERCUS SUBER CHLOROPLAST HAPLOTYPES MATCHES THE MIocene PALAEOGEOGRAPHY OF THE WESTERN MEDITERRANEAN


*) Dipartimento di Biologia Vegetale, Università di Roma “La Sapienza”, Piazzale Aldo Moro 5, 00185 Roma (Italy)
**) Consiglio Nazionale delle Ricerche, Istituto per la Protezione delle Piante, Via Madonna del Piano 10, 50019 Sesto Fiorentino (Italy)
*** Dipartimento di Tecnologie, Ingegneria e Scienze dell’Ambiente e delle Foreste, Università della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo (Italy)
**** Consiglio Nazionale delle Ricerche, Istituto di Genetica Vegetale, Via Madonna del Piano 10, 50019 Sesto Fiorentino (Italy)
***** Dipartimento di Biotecnologie Agrarie, Università degli Studi di Firenze, Via della Lastruccia 14, 50019 Sesto Fiorentino (Italy)

chloroplast DNA, Quercus suber, Neogene, geographic structure

Combining molecular analyses with geological and palaeontological data may reveal timing and modes for the divergence of lineages within species. The Mediterranean Basin is particularly appropriate for this kind of multidisciplinary studies, because of its complex geological history and biological diversity. Here, we investigated chloroplast DNA of Quercus suber populations in order to find possible relationship between their geographic distribution and the palaeogeography of the western Mediterranean domain. We analysed 110 cork oak populations, covering the whole distribution range of the species, by fourteen chloroplast microsatellite markers; among which eight displayed variation among populations. We identified five haplotypes, which distribution is clearly geographically structured. Results demonstrated that cork oak populations have undergone a genetic drift consistent with the Oligocene and Miocene break-up of the European-Iberian continental margin and suggested that they have persisted in a number of separate microplates, currently found in Tunisia, Sardinia, Corsica, and Provence, without detectable cpDNA modifications for a time span of over 15 Myr. A similar distribution pattern of mitochondrial DNA of Pinus pinaster supports the hypothesis of such long-term persistence, in spite of Quaternary climate oscillations and of isolation due to insularity, and suggests that part of the modern geographical structure of Mediterranean populations may be traced back to the Tertiary history of taxa.
EVOLUTIONARY PATTERNS OF QUERCUS SUBG. CERRIS IN THE ITALIAN PENINSULA

SIMEONE M.C.*, PAPINI A.**, VESSELLA F.*, PUDDU G.*, BELLAROSA R.*, SCHIRONE B.*

*) Dipartimento di tecnologie, ingegneria e scienze dell’Ambiente e delle Foreste (D.A.F.), Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo (Italy)
**) Dipartimento di Biologia Vegetale, Università degli Studi di Firenze, Via La Pira 4, 50121 Firenze (Italy)

Quercus, DNA, evolution, reticulation, hybrids, Italy

The evolutionary history of most plants cannot be fully represented by a phylogenetic tree. Rather, it would be more appropriate a phylogenetic network, in which there have been a large number of reticulate evolutionary events, such as hybrid speciation. Reticulate evolution implies gene flow between historically individuated lineages that nominally merit taxonomic distinction. The alternative process is cladogenesis without hybridization. Data from two or more molecular markers are expected to provide independent tests of phylogeny, especially if they are from different genomes and identify different kinds of mutations; they may thus interact to avoid incorrect reconstructions and to reveal discrepancies that could be interpreted as resulting from hybridization.

The complex evolutionary history of Quercus is still under debate, owing to the strong attitude of oaks to interspecific hybridization, and to their high morphological plasticity with regards to changing environmental conditions. Subgen. Cerris appears to be a characteristic taxon for Eurasia, and particularly interesting for the reconstruction of the evolutionary history of the whole genus Quercus in the Mediterranean region. However, phylogenetic patterns of variability within and among the individual species are still largely incomplete. As well, biogeographic diversity data on the Italian districts of the species range are unknown. New insights to infer speciation processes and intra-specific phylogeographic relationships in 5 Cerris taxa growing sympatrically in Italy (Q. cerris, Q. suber, Q. crenata, Q. trojana, Q. macrolepis) are here provided by means of plastid DNA and nuclear ribosomal ITS studies. Two additional oak species, in which introgression phenomena have been long documented (Q. coccifera, Q. ilex), have been used for comparison.

Evidence for a clear phylogeographical structure was detected with PCR-RFLP at 5 chloroplast loci, while Maximum Parsimony and Neighbor Joining analyses of the ITS sequence variation displayed patterns apparently unrelated with a congruent geographical distribution. Three chloroplast haplotypes and three ITS main lineages were identified in the Italian peninsula, stressing the importance of these territories for the evolutionary history of this group of species. Two divergent “Italian” haplotypes are highly shared within 5 oak taxa, and one ITS variant is basal to the ingroup, revealing sister relationships within the Cerris taxonomic group.

Hypotheses of lineage sorting of ancient DNA polymorphisms and of reticulate evolution of the whole species group have been inspected by use of the NeighborNet Analisys and preliminary data will be presented and discussed.
IN VITRO PLANT REGENERATION OF CAPER (CAPPARIS SPINOSA L.)

CARRA A.*, SIRAGUSA M.*, ABBATE L.*, SAJEVA M.**, CARIMI F.*

*) Institute of Plant Genetics – CNR, Research Division Palermo, Corso Calatafimi 414, 90129 Palermo (Italy)
**) Department of Botany Science, University of Palermo, Via Archirafi 38, 90123 Palermo (Italy)

plant regeneration, flower explant, in vitro culture, genetic fidelity, flow cytometric analysis

Caper (Capparis spinosa L.), a plant native to the Mediterranean Basin, is an extremely drought resistant plant. It is cultivated to harvest the unopened flowers or young fruits and used in many traditional dishes. Owing to these features, this shrub has become a valuable and specialized crop of great economic importance in the Mediterranean area both for local market and for export over the last decades.

Till now, propagation is mainly carried out by seeds, that generates high genetic flow, or by vegetative multiplication with several rooting problems.

In this work, a new technique to regenerate plants starting from flower explant is reported. In vitro plant regeneration was attempted using stigma, anthers and unfertilized ovules of unopened flowers collected from plants growing in the field. Plant regeneration was achieved from unfertilized ovules on MS medium supplemented with 3% sucrose and 13mM 6-benzylaminopurine (BAP). New individuals obtained from unfertilized ovules were used as source material for micropropagation and multiple shoots were obtained on MS medium supplemented with BAP and indole 3-butyric acid (IBA). Explants obtained in micropropagation step were used for rooting step under several hormones conditions. The best results were obtained when the explants were incubated in presence of 10mM IBA for six days in the dark and then transferred in hormone free medium and in the light.

New plants were vigorous, of good quality and presented phenotypic characters similar to mother plants.

To detect the genetic fidelity of regenerated plants, flow cytometric analysis and two different DNA-based techniques (ISSR and RAPD) were used.
MOLECULAR AND PHYTOCHEMICAL CHARACTERIZATION OF WILD HELICRYSUM ITALICUM (ROTH) G. DON


*) Department of Plant Production, University of Bari (Italy)
**) Department of Agro-Forestry and Environmental Biology and Chemistry, section of Genetics and Breeding, University of Bari (Italy)
***) Department of Pharmaco-Chemistry, University of Bari (Italy)

Helichrysum italicum (Roth) G. Don, AFLP, GC-MS, genetic diversity

Helichrysum italicum (Roth) G. Don, everlasting, is a native perennial herb in the Mediterranean area. It is a small aromatic shrub characterized by yellow flowers, growing on dry cliffs and sandy soil. The plant is mainly known for its anti-inflammatory, antiallergic and antimicrobial properties and its essential oil employed in phytotherapy.

Wild populations of everlasting represent a useful germplasm to select plants, producing high-value active compounds, which may be further introduced in specific breeding programmes and/or cloned in vitro.

The present research deals with the molecular characterization of spontaneous plants of H. italicum collected in different areas in South Italy (Puglia and Basilicata). Genetic variability of the wild plant samples by AFLPs markers has been assessed and correlated with observed phytochemical differences in the essential oil composition.

Genetic analysis allowed to group everlasting clones collected in South Italy into three different clusters. Phytochemical analysis revealed that the essential oils from those clones are mainly characterized by sesquiterpenes with α-selinene, β-selinene and γ-curcumene as the major components. Populations of H. italicum containing γ-curcumene are clustered in the same group, as well as clones with α-selinene, β-selinene.
IN VITRO AND IN VIVO EFFECTS OF ESSENTIAL OILS AGAINST PLANT PATHOGENS AND MYCOTOXIGENIC FUNGI

MORCIA C.*, TERZI V.*, DE BERNARDINI S.*, MALNATI M.**, FAccIOLI P.*

*) Istituto Sperimentale per la Cerealicoltura, C.R.A., Via S. Protaso 302, I-29017 Fiorenzuola d’Arda (Italy)
**) Unità di Virologia Umana, DIBIT, Istituto Scientifico San Raffaele, Via Olgettina 58,
20132 Milano (Italy)

in vitro fungal growth, Blumeria graminis, essential oils

The use of synthetic fungicides to control fungal diseases in crops can result in problems like environmental pollution, phytotoxicity and the selection of resistant pathogen populations. Therefore, alternative measures have been developed for crop protection, including biological agents, mineral salts and plant extracts. Among these, the complex mixtures of compounds, mainly monoterpenes and sesquiterpenes, that characterized the chemical composition of essential oils can potentially be considered as alternative natural fungicides.

In a previous study (Terzi et al, 2007), the antimycotic properties of Melaleuca alternifolia essential oil (TTO) and its principal components (terpinen-4-ol, gamma-terpinen and 1,8-cineole or eucalyptol) were evaluated in vitro on Fusarium graminearum, Fusarium culmorum and Pyrenophora graminea. All the tested fungi were susceptible to TTO and its components. More in details, TTO exerted a wide spectrum of antimycotic activity, but single TTO purified components were more active than the whole oil in reducing in vitro growth of fungal mycelium and, among the tested compounds, terpinen-4-ol was the most effective. Starting from these results, a set of essential oils, like thymol, eugenol, carvone, terpinen-4-ol, gamma-terpinen and eucalyptol were evaluated for their effects on a panel of twelve mycotoxigenic fungi belonging to different species and characterized by different host range. Moreover, the effect of TTO and other essential oils have been evaluated in field experiments to control Blumeria graminis in wheat and barley.

VARIATION OF ARTEMISININ CONTENT IN DIFFERENT CULTIVAR OF 
ARTEMISIA ANNUA L. DURING THE VEGETATIVE CYCLE

ABET M., NUNZIATA R., ASCIONE S., INTERLANDI G., SODANO E., DEL GAUDIO C., DI 
GIORGIO B.

CRA Istituto Sperimentale per il Tabacco, Via P. Vitello 108, 84018 Scafati (Italy) - 
massimo.abet@entecra.it

Artemisia annua L., artemisinin

Artemisia annua L. (Asteraceae), also known as Chinese herbal Qinghao, annual or sweet 
wormwood, is an annual herb originating from Asia. This plant has been used by Chinese herbalists 
for more than a thousand of years in the treatment of many diseases, such as skin diseases and 
malaria.

Artemisinin is an antimalarial compound present in Artemisia annua L. and is very effective 
against drug resistant Plasmodium species. Its therapeutic effect increases when used in 
combination with a series of semisynthetic derivatives like artemether, arteether, artelinic acid and 
sodium artesunate. Artemisinin is an oxidized sesquiterpene lactone with an “endoperoxide bridge”, 
that seems to be responsible of its antimalarial activity, and has mainly been detected in the aerial 
parts of the plant, in particular in the leaves and flowers.

As Artemisia has been proposed as an alternative crop in the areas of Campania liable to the 
conversion of tobacco, a preliminary study about the accumulation of artemisinin in the plant, 
during all vegetative stages, was performed, comparing three cultivars grown and two planting 
density. The trial was carried out in the experimental field of “CRA-Istituto Sperimentale per il 
Tabacco” in Scafati (SA), using a split plot design with two replicate blocks. Artemisia annua L. 
cultivars Crono, Eureka and Pericles were grown at the theoretic planting density of 111.000 and 
55.000 plants/ha. During the life cycle of the plant until flowering, every two weeks, the aerial parts 
(leaves and stalks) were harvested separately, weighed, dried in an air-forced oven at 60°C, 
grounded and analyzed by HPLC as described by Guo-Ping Qian and coworkers.

Preliminary data on the growth of the plant revealed that the planting density had a light 
positive effect on the development in height; the highest increase in plant dry weight has been 
observed between 90 and 105 days from the transplant. Moreover it was observed that the dry 
weight percentage of the leaves on the plant, for all varieties, decreased with the growth of the 
plant.

Artemisinin content in leaves increased during the vegetative cycle and revealed a different 
behaviour among cultivars, in particular Crono and Pericles reached the highest artemisinin values 
after 105 days from transplanting, while Eureka cultivar after 75 days. As regards the effect of 
planting density on artemisinin content the highest values were always observed at the lower 
density. Artemisinin content in the stalks was negligible.

This research was carried out with financial support of the European Community, 
fasce”.
DEVELOPMENT OF A TILLING SUNFLOWER POPULATION


*) Department of Agro-Forestry and Environmental Biology and Chemistry, section of Genetics and Breeding, University of Bari, Via Amendola 165/a, 70126 Bari (Italy)
**) Department of Management of Agro and Forestry Systems’ Mediterranean University of Reggio Calabria (Italy)
***) Department of Biology, Plant protection and Agro-Forestry Biotechnology, University of Basilicata, Potenza (Italy)

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 in vitamin E and 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. In many countries, considerable interest has been focused on the possibility of using vegetable seed oils as starting material for biodiesel. The advantages of this product are: low toxicity, high biodegradation and its renewable resources origin.

Many studies reported results about the manipulation of fatty acid composition for nutritional purposes (high-oleic mutant line obtained by chemical mutagenesis), but there is a lack of studies about the manipulation of fatty acid composition for industrial purposes.

Actually it is possible to use sunflower oil as a raw material to obtain biodiesel, only after the process of trans-esterification that is necessary to decrease the viscosity of sunflower oil. The manipulation of the bio-synthetic pathway of fatty acids by means of genetic tools involves modification of the fatty acid composition, which determines the chemical properties of the oil and its end use.

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

A stock of 7000 seeds of a open pollination standard cultivar of sunflower were treated with two concentration of EMS for different times in order to establish the acceptable percentage of germination after the treatment. Mutagenized seeds have been grown to obtain 2000 M_1 plants. To avoid ambiguities caused by chimerism of mutant plants in the first (M_1) generation, about 1200 M_1 plants were self-fertilized, and M_2 progeny from single seed descent was used for screening. The M_2 population was characterized for the presence of mutagenized phenotypes and is going to be analysed for a pilot screen on two genes to recover the mutation frequencies. The actual size of M_2 population will be implemented with a second stock of M_1 seeds, in order to obtain a complete population of 4000 plants.
IDENTIFICATION OF THE HOMEOLOGOUS, DROUGHT-RELATED TDDRIF1 GENE IN WILD ANCESTOR WHEATS, AEGILOPS SPELTOIDES AND TRITICUM URARTU

THIYAGARAJAN K.*,**, TESFAYE E.*,**, LATINI A.*

T.K. AND T.E. EQUALLY CONTRIBUTED TO THIS WORK

*) ENEA, Department of Biotechnology, AgroIndustry and Human Health, BIOTEC GEN, Via Anguillarese 301, 00123 Roma (Italy)
**) International Doctoral Programme in Agrobiodiversity, Scuola Superiore S. Anna, Pisa (Italy)

wheat, stress tolerance, DREB-related genes, agrobiodiversity

Drought is one of the most important abiotic stress that limits the productivity of wheat. Genetic improvement of crops for drought tolerance requires deep investigations and the exploration of the genetic variability of wild ancestor species. *Triticum urartu* and *Aegilops speltoides* are diploid wheat progenitor species characterized by AA and BB genomes, respectively.

Recently, DREB-related genes have been identified and characterized in several cereals. These genes play a key role in regulating plant abiotic stress response and can show a complex expression pattern, as the result of an alternative splicing of the transcripts.

We have screened the genomic DNA to identify the drought-related *TdDRF1* (Triticum durum Dehydration Responsive Factor 1) gene in various *Aegilops speltoides* and *Triticum urartu* accession lines, proceeding by different geographic areas. This gene codifies for two transcription factors and it is organized at genomic level in four exons and three introns. In the two functional transcripts, the exon 4 codifies for a nuclear addressing signal and the AP2 domain, a highly conserved region responsible for the cis-acting DNA binding.

Preliminary results strongly support the presence of the homeologous of *TdDRF1* gene in both genomes. In particular, the structure and sequence of these genes found in *Triticum urartu* (AA) seem to be closer to the modern durum wheat than *Aegilops speltoides* (BB).

Furthermore, the ongoing sequencing of different accessions will allow to assess the similarity to the *TdDRF1* gene and to identify some polymorphisms (SNPs) to evaluate their evolutionary significance. SNPs can be useful as molecular markers and genetic source for assisted breeding and selection.