NEXT-GENERATION DNA SEQUENCING AND SNP GENOTYPING TECHNOLOGIES ENABLE THE RAPID DEVELOPMENT OF A MARKER-ASSISTED BREEDING PLATFORM FOR WATERMELON


*) Institute of Plant Breeding, Genetics, and Genomics, The University of Georgia, Athens, Georgia, 30602, USA
**) Monsanto, Woodland, California, 95695, USA

Citrullus, single nucleotide polymorphism, next-generation DNA sequencing, marker-assisted selection

Modern watermelon (Citrullus lanatus ssp. lanatus) cultivars are products of intense breeding for increased sugar content, enlarged fruit, enhanced flavor, and decreased seed number. Domestication, selection for horticulturally important traits, and elite parent recycling have created population bottlenecks, dramatically narrowed genetic diversity among modern cultivars, and impeded forward genetic analyses and marker-assisted breeding (MAB) in elite x elite crosses. Moreover, limited genomic resources have been developed for watermelon, and previous analyses have been limited to unusual and exotic crosses. One of our goals was to develop the infrastructure needed for MAB in watermelon by targeting single nucleotide polymorphisms (SNPs) among modern cultivar alleles and developing the critical mass of SNP markers needed for genome-wide mapping and other applications in elite x elite crosses. First, several hundred simple sequence repeats (SSRs) were identified by reduced representation sequencing (RRS) of genomic DNA isolated from a single cultivar. Four hundred SSR markers were developed and screened for polymorphisms among 48 public and proprietary elite inbred lines. To thoroughly sample allelic diversity and identify common SNPs for applications in hybrid breeding programs, 18 elite inbred lines were selected for RRS using next-generation DNA sequencing technologies. Methylation-filtered genomic DNA libraries were produced and sequenced using next-generation technologies, assembled using MIRA, and mined for SNPs and other DNA polymorphisms using a custom SNP discovery pipeline. Genomic DNA sequences were produced by next-generation sequencing of RRS genomic DNA libraries, assembled using MIRA, and mined for SNPs and other DNA polymorphisms. Several thousand common SNPs were identified, filtered, and selected for validation and mapping using highly parallel SNP genotyping arrays. Three 1,536 SNP arrays were developed, yielded 3,400 validated SNPs, and enabled high-density genetic mapping of several hundred common SNPs and the assembly and orientation of complete linkage groups in several intraspecific populations. Next-generation DNA sequencing and SNP genotyping technologies mitigated long-standing technical problems and enabled the development of the infrastructure needed for the routine application of MAB approaches in elite x elite crosses in watermelon. We describe these and other MAB and genomics-assisted discovery strategies enabled by next-generation DNA sequencing and SNP genotyping technologies.
SOLANACEAE GENOMICS: MAYBE WE CAN

DI FILIPPO M.*, MASELLI V.*, TRAINI A., D’AGOSTINO N., FRUSCIANTE L., CHIUSANO M.L.

Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples “Federico II”, Via Università 100, 80055 Portici, Italy

*authors contributed equally to this work

Solanaceae genomics, tomato genome project, gene /repeat content, orthologous genes

The need to enhance our knowledge on the genetic mechanisms which determine Solanaceae diversification and adaptation to extremely different environments has led scientific efforts to be gathered under the International Solanaceae (SOL) Genome Project.

The long-term goal of the SOL Consortium is to exploit the information generated by the tomato genome sequencing project to analyse the genome organization, the functionality and the molecular evolution of the entire Solanaceae family by using comparative approaches.

The tomato (Solanum lycopersicum) genome project (Mueller et al., 2009) is currently focused on the BAC-by-BAC (Bacterial Artificial Chromosome) sequencing of the euchromatin region (~250 Mb), which is also expected to be gene richer (Peterson et al., 1996).

Solanaceae transcriptome data which are part of the Italian Solanaceae Platform (ISOL@, Chiusano et al., 2008), integrated with the available genome sequences, allowed us to perform preliminary investigations on structural features and on evolutionary relationships emerging from the current draft of the tomato genome.

The screening of the gene and the repeat content per BAC revealed: (i) large variability in both repeat and gene coverage along chromosomes; (ii) in spite of this variability, repeat- or gene-richer BACs generally were organized as blocks; (iii) candidate pericentromeric regions on the basis of the repeat/gene relative content; (iv) not all the repeat rich blocks were associated to the heterocromatic pericentromere; (v) repeat-rich blocks, including coding regions with somehow interesting functionalities. Possible associations of the BAC composition to chromatin packaging are being also considered. In addition, while waiting for an official annotation by the Tomato Genome Sequencing Consortium, we defined a first set of tomato genes exclusively based on EST (Expressed Sequence Tag) evidence (D’Agostino et al., 2007). The gene set was also used to identify putative orthologous genes based on the identification of reciprocal best BLAST hits with the protein complement from A. thaliana, O. sativa cv. japonica, V. vinifera (PN40024 and ENTAV111 clones), respectively. A common gene repertoire was defined among all the plant species considered, and structure relationships were investigated.

In conclusion, although the tomato genome currently available is only 120 Mb, an integrated bioinformatics platform and novel computational strategies permitted: i) to reveal a typical design...
of the tomato genome structure; ii) the definition of a preliminary gene set for comparative analysis within available plant genomes and iii) to set up an useful framework for the analysis of novel Solanaceae genomes. This makes us confident that maybe we can now move towards the challenge of the SOL view.
INTROGRESSION BREEDING IN EGGPLANT (SOLANUM MELONGENA L.) BY COMBINING BIOTECHNOLOGICAL AND CONVENTIONAL APPROACHES


*) CRA-ORL, Unità di Ricerca per l’Orticoltura, via Paulese 28, 26836 Montanaso L. (LO), Italy
**) CRA-ORA, Monsampolo del Tronto (AP), Italy
****) CRA-ORT, Pontecagnano (SA), Italy
****) CRA-IAA, Milano, Italy

interspecific hybridization, resistance genes, Solanum spp, Fusarium wilt

Breeding programs, aimed at the introgression of innovative traits into the eggplant (Solanum melongena L.) gene pool through both sexual and somatic hybridization, have been set up in the middle of 1990’s in the frame of international (EC) and national (MiPAAF) research projects. A sexual interspecific hybrid was obtained with the wild species S. sodomaeum (syn. S. linneanum) carrying tolerance to verticillium wilt, drought and salt. Tetraploid somatic hybrids were obtained by electrofusion of eggplant and S. aethiopicum gr gilo or S. integrifolium protoplasts, both allied species being a source of resistance to wilts caused by Fusarium oxysporum and Ralstonia solanacearum, and dihaploids were obtained through anther culture. Molecular, biochemical and phenotypic analyses demonstrated that partial genetic recombination occurred between the genome of eggplant and those of the allied species. Advanced introgression lines, phenotypically indistinguishable from the recurrent genotypes were obtained through 6-8 backcross cycles and selection, followed by selfing and/or anther culture to obtain pure lines. Some seed companies joined the last steps of the breeding program in view to exploit these genetic materials to release commercial F1 hybrids suited for both open field or greenhouse cultivations. Crosses among introgression lines from different allied species have been carried out to cumulate useful traits such as Fusarium resistance and Verticillium tolerance, and molecular analyses confirmed that COS and SSR of allied species were still present in the advanced introgression lines. The introgressed Fusarium resistance trait is controlled by the dominant resistance Rfo-sal1, of which alleles are present in S. aethiopicum and S. integrifolium. BSA analysis enabled to develop codominant CAPS markers tightly linked to the Rfo-sal1. Our best selected lines, phenotypically similar to the most common Italian typologies, are resistant to Fusarium, highly tolerant to Verticillium, fertile and productive, the fruits display good colorations of skin and flesh, and firmness. Biochemical analyses revealed high differences between eggplant and its allied species in chlorogenic acid, total polyphenol, glycoalkaloids, anthocyanins and lipid fatty acids contents as well as PPO activity and antioxidant potential. Total amount of glycoalkaloids in allied species is close to or exceeds the recommended safety value in plant, however we recovered introgressed lines with glycoalkaloid content analogous to that of the recurrent eggplant genotypes.

Functional studies enabled to identify a number of genes putatively involved in the resistance to the wilting fungi. The development of a dense genetic map, based on SSR, AFLP, COS and SNPs is in progress to fine mapping the Rfo-sal1 locus and to find markers associated with agronomically important traits.
CONSTRUCTION OF A REFERENCE LINKAGE MAP FOR GLOBE ARTICHOKE


*) DIVAPRA, Plant Genetics and Breeding, University of Torino, Italy
**) DACPA, Scienze Agronomiche, University of Catania, Italy
***) Institute for Plant Breeding, Genetics, and Genomics, University of Georgia, USA

**Cynara cardunculus, genetic mapping, linkage analysis, EST derived microsatellite**

Globe artichoke (Cynara cardunculus var. scolymus L. 2n=2x=34), a member of the Asteraceae (=Compositae) family, is an important crop for the Mediterranean basin agricultural economy. In spite of its commercial relevance and nutraceutical properties, its genome structure has been elucidated to a limited extent. To move towards modern breeding strategies it is compulsory to establish genetic maps and identify the genetic bases of key traits of interest. The species is highly heterozygous and suffers marked inbreeding depression when forced to self-fertilize; thus, a two-way pseudo-testcross represents the optimal strategy for linkage analysis.

We produced three F1 progenies obtained by crossing a globe artichoke clone of ‘Romanesco C3’, as female parent, with three pollen sources: a genotype of globe artichoke ‘Spinoso di Palermo’ (Progeny 1); one of cultivated cardoon (var. altilis, Progeny 2) and one of wild cardoon (var. sylvestris, Progeny 3). The first globe artichoke genetic maps were developed by genotyping Progeny 1 and applying a variety of PCR-based marker platforms. Since more markers were needed to saturate the maps, we developed a further wide set of SSR markers based on the ESTs (expressed sequence tags) of globe artichoke, recently made available by the Composite Genome Project (CGP; http://compgenomics.ucdavis.edu/). Using a custom bioinformatic pipeline, 36,321 ESTs were assembled into 19,055 unigenes (6,621 contigs and 12,434 singletons), annotated, and mined for perfect SSRs. We identified 4,219 perfect repeats in 3,308 unigenes (1 SSR per 3.6 kbp), designed genotyping primers for 2,311 SSRs, and tested primers for 300 loci. Among them 176 to 198 (75 to 84%) were polymorphic in the three mapping populations.

As a result we constructed new linkage maps based on Progeny 2; the population was genotyped using mainly AFLPs and microsatellites; about one thousand markers suitable for map construction were identified, which were assigned to 18 major linkage groups in both maps. The male map consisted of 274 loci spanning 1428 cM, with a mean inter-marker distance of 5.5 cM while the female one was built with 475 loci spanning 1.688 cM and a marker density of 3.7 cM; the latter will provide a favorable property for QTL scanning; furthermore, as about 160 mapped markers (34%) belong to the gene space, has an additional values as functional map and might represent an important genetic tool for candidate gene studies in globe artichoke. The two maps shared 66 co-dominat loci, which allowed for the alignment of all the LGs as well as the construction of an integrated SSR-based linkage map, which includes 227 microsatellite and SNP markers targeting genes involved in the synthesis of caffeoylquinic acids. Other maps, based on Progeny 3, are currently under construction and markers shared by the three F1 progenies will be used as anchor points for map integration and comparative genetic analyses.
A high phenotypic variation has been observed for important agronomic traits in all the three F1 populations which, being *C. cardunculus* easily vegetatively propagated, are immortalized. These populations are grown in contrasting environments making the identification of quantitative trait loci more efficient and reliable.
Identification and transfer of QTLs controlling ascorbic acid content in tomato fruit

DI MATTEO A., SACCO A., ANACLERIA M., TROTTA N., BARONE A.

Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples Federico II, Via Università 100, 80055 Portici, Italy

tomato fruit quality, transcriptomic analysis, introgression lines, QTL pyramiding

Tomato fruit is a precious source of nutritional compounds like ascorbic acid (AsA), which can help to protect against human diseases such as cancer and cardiovascular ones. In addition, it plays a crucial role in plants as enzymatic co-factor and antioxidant. Therefore, one objective of tomato breeding is to increase AsA content in fruit and this requires a deep understanding on the genetic control of its synthesis and storage.

The purpose of the present work was to identify QTLs that can increase AsA content in tomato fruit with the final aim of pyramiding them into cultivated varieties. Suitable genetic resources exist in tomato to help dissecting QTLs, which are the introgression lines, i.e. a collection of homozygous lines, of which each contains marker-defined segments of the wild genome in an uniform cultivated genetic background. Fifty *S. pennellii* introgression lines were screened for AsA content in fruit during three years (2006, 2007, 2008) and three of them were selected since they significantly differed from the cultivated genotype M82 over all the three years. Two of them showed a higher AsA content (IL7-3, IL12-4), while one showed a lower content (IL10-1). RNA was extracted from fruit collected from the IL12-4 line during the years 2007 and 2008 using three plant replicates per year. It was hybridized on the Combimatrix 90k TomatArray 1.0, synthesized at the University of Verona. The microarray slide included 20200 4x-replicated in situ synthesized probes, designed to specifically match Tentative Consensus (TCs) retrieved from the TIGR database *S. lycopersicum* Gene Index Release 11.0 (June 21, 2006). A group of over-expressed and under-expressed probes in the IL12-4 vs. M82 comparison were validated by qPCR and a model explaining the higher AsA content in the IL 12-4 fruit was proposed. Using a bioinformatic approach, many differentially expressed TCs were also localized on the tomato chromosome 12 in the region of introgression 12-4, and this allowed the model hypothesized to be further on supported. Furthermore, the study of genetic control of AsA content is in progress also for the IL7-3, by using the same transcriptomic and bioinformatic analyses.

Finally, a breeding scheme was started to introgress the IL12-4 region in various cultivated tomato genotypes and to pyramid in the same genotype more QTLs that increase AsA in fruit, some deriving from IL12-4 and others from IL7-3. In order to speed up this breeding effort, polymorphic markers spanning the 12-4 and 7-3 regions have been selected, and this will allow the size of introgressed regions to be reduced to those carrying the putative candidate genes involved in the AsA control.
The genus Asparagus consists of around 150 species found as herbaceous perennials, tender woody shrubs and vines [1], and is classified into three subgenera (Asparagus, Protasparagus and Myrsiphyllum) according to Clifford and Conran [2]. The species of the first subgenus are dioecious, with unisexual flowers, while the second and the third subgenera include only hermaphroditic plants. In the Asparagus genus some species have economic value as ornamentals (Asparagus plumosus, A. densiflorus, A. virgatus, A. myriocladus and A. retrofractus) or are noted for their medicinal properties (A. racemosus, A. verticillatus, A. adscendens and A. curillus). The wild A. maritimus and A. acutifolius are known to be used in some diets, but the most economically important Asparagus species is garden asparagus (A. officinalis) which is a highly prized vegetable. Asparagus officinalis L. is an important horticultural crop, grown in regions with a temperate climate. The Eastern Mediterranean and Minor Asia are considered by Ellison [1] to be the centre of origin of this species. Asparagus (Asparagus officinalis) plants are naturally dioecious, either male or female, with 2n=2x=20 chromosomes and a haploid genome size of 1323 Mb [1].

In order to conduct marker-assisted selection (MAS) in Asparagus we generate the first broad survey of EST-SNPs (Expressed Sequence Tag-Single Nucleotide Polymorphisms) in A. officinalis specie; more than 200,000 Asparagus officinalis ESTs were sequenced and assembled starting from two GSFLX 454 pyrosequencing runs on two parental genotypes sharing a higher part of their genetic background and divergent at least for Puccinia asparagi resistance trait (male genotype named G190 as resistant, female genotype named 1770 as susceptible ones). We developed an efficient computational SNPs mining pipeline based on a sequence polymorphisms detection tool, finding more than 1700 putative G190/1770 SNPs within expressed sequences. Stringent post-processing reduced this number to 284 putative SNPs and a panel of these were successfully validated by Sanger sequencing.

Expressed sequence tag databases are a valuable resource of gene-rich DNA sequence information for a species, additionally that can be mined for the development of genetic markers as SNPs.

Once discovered, SNPs can be converted into genetic markers that can be inexpensively assayed in a high-throughput manner and it can be use for generate very dense genetic maps [3].
Until now, there are few deposited *Asparagus* EST sequences, especially for *A. officinalis*, and the repository of a massive number of those from our study can be useful for genome sequence annotation and, especially, for continued advancement of *Asparagus* genomics research.


FINE MAPPING OF Ol-qtl2, A QTL CONFERRING RESISTANCE TO TOMATO POWDERY MILDEW

FAINO L.*, HOUSHYANI HASSANZADEH B.*, VERZAUX E.*, FRUSCIANTE L.**, BAI Y.*

*) Plant Breeding Laboratory, Wageningen University and Research centre, Droevendaalsesteeg 1 6708 PB Wageningen, The Netherlands
**) Department of Soil, Plant, Environmental and Animal Production Sciences University of Naples “Federico II”, Via Università 100, 80055 Portici, Italy

resistance gene, plant disease, powdery mildew, resistance QTL

Tomato (Solanum lycopersicum) is one of the most important vegetable. Several pathogens are able to infect this specie. Due to the lack of genetic diversity in the cultivated tomato, it is necessary to discover resistance genes by exploring wild tomato species. The tomato powdery mildew is an important disease and one causal agent is Oidium neolycopersici. The first observation of tomatoes infected by O. neolycopersici was in the late 80’s. Although this disease is relatively new, many resistance genes (R-gene) have been identified in wild tomato species, including Ol-1 and Ol-3 identified from S. habrochaites, the Ol-4 from S. peruvianum and three quantitative traits loci (QTL) from S. neorickii. In the last few years efforts has been addressed to the introgression of resistance QTLs (R-QTL) into modern cultivars because it is assumed that the resistance conferred by R-QTLs is more durable comparing with the major R-gene mediated resistance. Three QTLs (Ol-qtl1, Ol-qtl2 and Ol-qtl3) have been discovered in S. neorickii (G1.1601), which confer resistance to O. neolycopersici. Ol-qtl1 is located on the long arm of the chromosome 6 and Ol-qtl2 and Ol-qtl3 are liked and mapped on the short arm of chromosome 12. The aim of this research is to fine-map and to clone the two QTLs on chromosome 12.

For fine-mapping, two BC2S1 populations (pop222 and pop242), which are derived from a cross between S. lycopersicum cv. Moneymaker and S. neorickii G1.1601, have been used. Pop222 harbors the S. neorickii alleles of Ol-qtl2 and Ol-qtl3 in a chromosomal region flanked by markers T0659 and TG111. Twenty-one co-dominant markers, spanning the Ol-qtl2 and Ol-qtl3 loci, were generated and applied on 168 individuals of pop222. The results confirmed one QTL, Ol-qtl2 which was mapped in a two-LOD supported interval of 6cM flanked by markers c2At2g06530 and imp3. The highest LOD value was associated with marker CT129. Five BC2S1 individuals, which were recombinants in the Ol-qtl2 region, were selected and selfed to produce BC2S2 families. By analyzing these five BC2S2 families (n=40, per family) with markers and disease tests, Ol-qtl2 could be located in a chromosomal region between two markers A and B at a genetic distance of about 6.5cM. Further, another BC2S1 population (pop242) was used to verify the results obtained in pop222. From 580 BC2S1 plants, twenty-four were selected as recombinants between the markers A and B and selfed to generate BC2S2 families. By analyzing these five BC2S2 families (n=40, per family) with markers and disease tests, Ol-qtl2 could be located in a chromosomal region between two markers A and B at a genetic distance of about 6.5cM. Further, another BC2S1 population (pop242) was used to verify the results obtained in pop222. From 580 BC2S1 plants, twenty-four were selected as recombinants between the markers A and B and selfed to generate BC2S2 families. By using the recombinant BC2S2 families (n=30 per family), the localization of Ol-qtl2 flanked by markers A and B was confirmed. Moreover, all the four BC2S2 families were genotyped with new BAC specific markers that have been generated from the tomato sequencing project. The results obtained with the new BAC specific markers, localized the Ol-qtl2 locus between markers C and D at a physical distance of about 100Kb. Within the 100Kb 15 coding sequences (CDS) were identified by using FgeneSH, of which six showed
homology to genes involved in resistance. All of these six CDSs were expressed in both parental lines.

In summary, we confirmed the presence of Ol-qtl2 on the short arm of chromosome 12, which is located between the markers C and D in a physical distance of 100Kb where several CDS were identified as candidate genes. In order to clone Ol-qtl2, we have generated a BAC library of S. neorickii G1.1601. Our next step is to identify BACs in the chromosomal region where Ol-qtl2 is located. We expect that sequence comparison between the susceptible and resistant parental lines will facilitate the cloning of Ol-qtl2.
EVOLUTION OF FLOWERING TIME AND FRUIT QUALITY TRAITS IN TOMATO

FALCONE G.°, FANTINI E.°, GIULIANO G.

ENEA, Casaccia Research Center, Via Anguillarese 301, 00123 Roma, Italy

° These authors contributed equally to this work

The genome of cultivated tomato (S. lycopersicum) has a limited sequence variation due to bottlenecks during domestication and breeding; however, the study of genetic diversity between tomato and related wild species could provide useful tools for the breeding of agronomically useful characters. We took a candidate gene approach to identify genetic differences responsible for the variability of two traits: the photoperiodic flowering response and the different colour of ripe berries. S. lycopersicum ecotypes and closely related wild tomato species were selected. The CRYPTOCHROME and CONSTANS gene families, controlling flowering time in Arabidopsis, have been completely sequenced, but up to now no mutations discriminating day-neutral from short-day species have been identified. Concerning berry colour, we studied the carotenoid biosynthetic pathway. The sequencing of genes from PSY down to LCY-e (alpha-branch) and CHY1/CHY2 (beta-branch) is complete. The sequence analysis has highlighted the presence of numerous mutations that differentiate the colour-fruited species from the green-fruited ones. Some non-synonymous substitutions are candidate to be hypomorphic alleles.
TOMATO GENOME SEQUENCING


*) Department BAS, ENEA, Casaccia Research Center, Via Anguillarese 301, 00123 Roma, Italy
**) Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples "Federico II", Via Università 100, 80055 Portici, Italy
*****) ENEA, Trisaia Research Center, S.S. Ionica - Km 419.5, 75026 Rotondella (MT), Italy
****) CRIBI Biotechnology Centre and Department of Biology, Univ. of Padova, Via U. Bassi 58/B, 35131 Padova, Italy
******) CNR – Institute of Plant Genetics – Portici, Via Università 133, 800525 Portici, Italy

tomato, chromosome 12 sequencing

Tomato (Solanum lycopersicum) is an economically and nutritionally valuable crop and constitutes a model plant for the Solanaceae family. Its genome encodes approx. 35,000 genes, which are largely grouped in contiguous euchromatic regions corresponding to approx. 25% of the 950 Mb genome. An international project is under way to sequence the euchromatic DNA on a BAC-by-BAC strategy (http://sgn.cornell.edu/about/tomato_sequencing.pl). Italy is sequencing chromosome 12 and providing mapping and bioinformatic tools to the international effort. Seed BACs are selected for the presence of genetic markers and validated via genetic (Introgression Line) and cytogenetic (FISH) mapping. Each sequenced seed BAC is then extended into a minimum tiling path. Approx 41% of the euchromatic portion is publicly available (43% on Chromosome 12). A bioinformatic platform has been built to provide a preliminary annotation of the genome. An additional effort, to obtain a draft WGS sequence with Next Generation sequencing, is under way.
UNDERSTANDING THE GENETIC CONTROL OF ANTIOXIDANT COMPOUNDS IN TOMATO FRUIT THROUGH GENOMICS TOOLS

RUGGIERI V., GRECO B., LOMBARDI N., VASCO M., BARONE A., DI MATTEO A.

Department of Soil, Plant, Environmental and Animal Production Sciences, School of Biotechnology, University of Naples “Federico II”, Via Università 100, 80055 Portici, Italy

antioxidant, introgression lines (ILs), transcriptional profile, TILLING

Antioxidant compounds in plant play a crucial role in controlling the level of reactive oxygen species (ROS). These metabolites are important in human diet to prevent cancer and other diseases. Tomatoes are one of the most valuable sources of antioxidants, such as carotenes (mostly lycopene and alpha-carotene, a precursor of vitamin A), ascorbic acid (AsA), and phenolic compounds (flavonoids and hydroxycinnamic acid derivatives). The aim of this work is to understand the genetic control for antioxidant content in tomato fruit, particularly ascorbic acid and phenols, by using genomics-based strategies.

In the first approach used, a population of introgression lines (ILs) of Solanum pennellii in the S. lycopersicon var. M82 genomic background was grown in a cold greenhouse, for two consecutive years, using three to five plant replicas per line each year. Fruit were harvested at red ripe stage and were assayed for phenol content. A QTL for increased phenols accumulation was detected in the IL 7-3. To better understand the genetic control of phenols synthesis and accumulation a microarray comparative analysis was performed. The RNA isolated from IL 7-3 and M82 fruit was hybridized on the 90k Combimatrix TomatArray 1.0 provided by the University of Verona. Preliminary results provide evidence of 188 differentially expressed transcripts, 53 up-regulated and 135 down-regulated. Currently, the single differentially expressed genes identified by microarray analysis are being validated by the qPCR approach.

The second genomics approach used aimed to functionally characterize candidate genes controlling antioxidants levels in tomato fruit. At this purpose, a tomato TILLING (Targeting Local Lesion IN Genomes) platform was used in collaboration with Metapontum Agrobios. In particular, the population was screened in order to find mutations in the genomic sequence of a transcription factor we previously showed to be involved in antioxidant accumulation in tomato fruit. Five point-mutations were identified and actually molecular and physiological analysis of mutants are in progress. In the future, other candidate genes involved in antioxidant control in tomato fruit will be characterized using the same TILLING population.
A WIDE TOMATO DISEASE GENES SCANNING FOR R GENE MARKERS DEVELOPMENT

CARLI P., SANSEVERINO W., FERRIELLO F., ERCOLANO M.R.

Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples “Federico II”, Via Università 100, 80055 Portici, Italy

in silico analysis resistance gene, tomato genome, SNPs, multiplex

Plant disease resistance genes (R-Genes) are an important class of genes from which a subset are well characterized at the molecular level. These genes play a key role in the recognition of the products of avirulence (Avr) genes from pathogens and in the activation of plant defence responses. In the Solanaceae family, 29 R-genes have been isolated and well studied. Starting with this genes pool, we were able to predict 257 putative R genes in tomato genome. In silico information were used to characterize some R regions by SNPs. Recent development in discovering SNPs within the cultivated species of tomato allowed the use of these markers in tomato breeding programs.

In this study such markers have been employed to detect R genes polymorphisms among closely related individuals within species (e.g. between elite cultivars) or between S. lycopersicum and closely related species. A SNaPshot multiple single nucleotide interrogation technique was adapted to identify simultaneously SNPs in genes involved in the tomato stress biotic resistance. In the SNaPshot reaction, the primers for single base extension (SBE) were designed to bind immediately adjacent 5’ to the SNP. During thermal cycling, primers were labeled with a distinct fluorophore, revealing different SNP haplotypes. To facilitate detection of several polymorphisms in a single run, the length of the extension primers was adjusted to a distinct size by addition of a poly (dA) code to their 5’ site. Each fluorescent ddNTP allowed a different wavelength, which translated into a specific colour for each base. In conclusion, we developed a rapid, accurate, and thoroughly validated method to discover new sequences putative for resistance function in tomato genome. Results have been used to perform simultaneous genotyping of several polymorphisms in different resistance pathogen genes. This assay provides a useful tool to identify simultaneously more polymorphisms in multiple genes and to screening large populations.
TOMATO IONOMIC APPROACH FOR FOOD FORTIFICATION AND SAFETY


*) University of Naples "Federico II", School of Biotechnology, Department of Soil, Plant, Environmental and Animal Production Sciences, Via Universitá 100, 80055 Portici (NA), Italy
**) ENEA Italian national Agency for New Technologies, Energy and the Environment, Portici - Research Centre, Via Vecchio Macello, 80055 Portici (NA), Italy

ionomic, Solanum lycopersicum, Introgression Lines, food fortification, food safety

Food fortification is an issue of paramount importance for people living both in developed and in developing countries. Among substances listed as "nutriceuticals", essential minerals have been recognised for their involvement in several healthy issues, involving all ages. In this frame, food plants are playing a pivotal role since their capability to compartmentalise ions and protein-metal complexes in edible organs. Conversely, the accumulation of high metal levels in those organs may lead to safety problems. In the recent years, thanks to the availability of new and improved analytical apparatus in both ionic and genomic/transcriptomics areas, it is became feasible to couple data coming from plant physiology and genetics. Ionomics is the discipline that studies the cross-analysis of both data sets. Our group, in the frame of GenoPom project granted by MiUR, is interested to study the ionomics of tomatoes cultivars derived by breeding programmes in which wild relatives have been used to transfer several useful traits, such as resistance to biotic or abiotic stresses, fruit composition and textiture, etc. The introgression of the wild genome into the cultivated one produces new gene combinations. They might lead to the expression of some traits, such as increased or reduced adsorption of some metals and their exclusion or loading into edible organs, thus strongly involving the nutritional food value. Our final goal is to put together data coming from ions homeostasis and gene expression analyses, thus obtaining an ionomic tomato map related to ions absorption, translocation and accumulation in various plant organs, fruits included. To follow our hypothesis, we are studying the ionome of *Solanum lycopersicum* cv. M82 along with 76 Introgression Lines (ILs) produced by interspecific crosses between this cultivar and the wild species *S. pennelli*. These ILs are homozygous for small portions of the wild species genome introgressed into the domesticated M82 one. They are used as a useful tool for mapping QTL associated with many traits of interest. It is worthy to note that, until now, little information is available on QTL for ions accumulation in tomato. Moreover, as our knowledge, effects of new gene combinations in introgressed lines on ions uptake related to food safety have not been extensively studied. In this presentation we show results coming from the ionome analysis, carried out on *S. lycopersicum* M82 and several ILs. Plants were grown in pots in a greenhouse and watered with deionised water Thirty day-old plants were left to grow for 15 days in the presence of non-toxic concentration of Cd, Pb, As, Cr and Zn given combined. Leaves of all plants were then harvested and stored at -80°C for ionome and gene expression analyses. Preliminary results of ionome analysis of *S. lycopersicum* M82 and several ILs, carried out using an ICP-MS, showed that traits correlated to toxic metals and micronutrients accumulation in apical leaves were significantly
modified in response to specific genetic backgrounds. Those results are perhaps due to the introgression of traits linked to uptake, translocation and accumulation of useful and/or toxic metal into plant apical leaves and to interactions of the wild type introgressed genomic regions with the cultivated genome. Also, data are shown on the identification and isolation of *Solanum* gene sequences related to ions uptake, translocation and accumulation, useful for further real-time gene expression evaluation in both cultivated and ILs during the treatments with the above-mentioned metals.
PROTEOMICS AND FUNCTIONAL GENOMICS APPROACHES TO EXPLORE CHLOROPLAST-CHROMOPLAST TRANSITION IN TOMATO FRUIT


*) Dipartimento di Biotecnologie Università di Verona, 37134, Italy
**) Institute of Plant Genetics – CNR, Research Division Portici, Portici, 80055, Italy
***) ENEA, Trisaia Research Center, S.S. 106 Jonica, Rotondella, Matera, Italy
****) Italian National Agency for New Technologies, Energy and the Environment (ENEA), Casaccia Research Center, Rome, Italy

RNAi, protease, stress tolerance, plastids differentiation, heat shock proteins

Chloroplast to chromoplast transition has been investigated in tomato (*Solanum lycopersicum*) fruit.

Our results reveal that only “green” fruit have photosynthetically active tissues releasing O2, while low PSII activity was measured also in “orange” fruit, but not in “red” fruit. In order to explore changes at protein level during chloroplast-chromoplast transition, we applied a detailed proteomic analysis on plastids purified from tomato berries at four different developmental stages. Using a MudPIT (Multidimensional Protein Identification Technology) LC/MS proteomic technique we identified more than 400 different polypeptides present in the four ripening stages. Our results show a strong decrease of photosynthetic proteins during fruit maturation, while heat shock proteins, chaperonins and plastid lipid associated proteins (PAP) increase. Together with an accumulation of stress related proteins we also detected an increase of Reactive Oxidative Species in plastids during chloroplast-chromoplast transition. Interestingly, the early steps of photosynthetic machinery reduction during fruit maturation affect the same photosynthetic proteins (LHClII, CP24, PSI-LHCII) decreased in leaves during acclimation to high light conditions. These results suggest a common mechanisms of photosynthetic proteins turnover modulation during chloroplast-chromoplast transition and high light acclimation. In order to better understand the role of the differentially expressed proteins during fruit ripening, we started a functional study by RNA interference (RNAi). For this purpose we are using hairpin RNAi (hpRNAi) vectors based on the Gateway™ recombinational cloning which facilitates high-throughput applications. In particular, hpRNAi constructs under the control of the constitutive 35S promoter were generated to induce gene silencing of HSP, PAP and protease differentially expressed proteins.
ANALYSIS OF DIFFERENTIALLY-EXPRESSED GENES CAUSED BY THE RFO-SA1 RESISTANCE LOCUS IN EGGPLANT BREEDING LINES INOCULATED WITH FUSARIUM OXYSPORUM F. SP. MELONGENAE AND VERTICILLIUM DAHLIAE

BARBIERATO V., RINALDI P., CAPONETTO G., LEONE E., TOPPINO L.

CRA-ORL, Unità di Ricerca per l’Orticoltura, Montanaso Lombardo, Italy

resistance genes, Solanum melongena, Plant-pathogen interaction, PCR select, S. aethiopicum

The two fungal wilts caused by Verticillium dahliae and Fusarium oxysporum f.sp melongenae are among the most serious diseases harming the eggplant production both in greenhouses and open field cultivation.

Interspecific somatic hybridization is considered a valid option to transfer disease resistance traits from the allied species into Solanum melongena L. genome in case of sexual incompatibility.

The resistance locus Rfo-Sa1 was introgressed from allied species into cultivated eggplant through crossing the dihaploids obtained from anther culture of the tetraploid somatic hybrids [S. melongena + S. Aethiopicum] and [S. melongena + S. Integrifolium] with recurrent eggplants. Identification of differentially-expressed genes involved in the plant-pathogen interactions between Fusarium and/or Verticillium dahliae and these new eggplant introgressed breeding lines was carried out. The resistant backcrossed lines were separately infected with Fusarium, Verticillium and both fungi together, while roots dipping in water was used as mock inoculation. Three libraries of genes involved in the plant-pathogens interaction were created by PCR-select comparison between the mRNA from inoculated and mock inoculated plantlets. The subtractive enrichment of the resulting samples lead to clone in E. Coli 1000 total differentially expressed sequences for each library. These clones were confirmed through colony Northern analysis.

Up to now, we chose from the three libraries the most promising 800 cDNAs for sequencing analysis. Cleaned sequences were subjected to Blast analyses, using the BlastN homology search tool, employing the NCBI (The National Centre for Biotechnology Information), SGN (SOL Genomics Network) and MiBASE (MicroTom Database) databases.

All the analysed sequences were grouped into three major categories: clones aligned with sequences of known function, clones aligned with sequences of unknown or hypothetical roles and clones with no alignment with sequences in the database.

The clones belonging to the group with known function were classified into the following metabolic groups: primary metabolism and photosynthesis; DNA replication, regulation and expression; translation; protein synthesis, degradation and modification; protein transport and translocation/membrane associated; cell wall, division and cytoskeleton; defence mechanism/secondary metabolism; stress induced proteins.

Considering the assigned categories, we evaluated and compared the different expression profiles distinctive of each fungal infection alone, and also of the mixed infection. Of particular interest will be genes differently associated with response to pathogen among the three types of
infection. Putative genes of interest will be validated by qRT-PCR and some of them will be further characterized.
A NEW INTRASPECIFIC AFLP-BASED MAP OF EGGPLANT

BARCHI L.*, STÀGEL A.*, PORTIS E.*, ROTINO G.L.**, TOPPINO L.**, LANTERI S.*
*) DIVAPRA, Genetica Agraria, Università di Torino, Grugliasco (TO)
**) CRA-ORL Unità di Ricerca per l’Orticoltura, Montanaso Lombardo (LO)

Solanum melongena, genetic linkage map, AFLP, SNPs, Fusarium resistance

Eggplant (Solanum melongena L., 2n=2x=24) is a member of the Solanaceae family, but unlike most of the solanaceous crop species, it is endemic to the Old World. In spite of its widespread cultivation, and its nutritional and economic importance, its genome has not as yet been extensively investigated. At present two interspecific and one intraspecific linkage maps based on F2 progenies are available, but the reduced size of the mapping populations as well as the limited number of markers applied, severely limit their precision.

We developed a new eggplant intraspecific genetic linkage map by crossing the lines “305E40” and “67/3”. The first one is a doubled haploid line obtained by anther culture of an advanced introgression line (BC7) derived from the somatic hybrid between Solanum aethiopicum gr. gilo (+) S. melongena cv. Dourga, it is characterized by elongated fruit and resistance to Fusarium oxysporum f.sp. melongenae. The line “67/3” derives from the cross between S. melongena cv. Purpura X S. melongena cv. CIN2, followed by 7 cycles of selfing, is characterized by round fruit type and susceptibility to F. oxysporum.

An anther-derived DH and a sexual F2 populations were obtained from the hybrid “305E40 x 67/3” and a sample of 93 individuals from both the populations was genotyped with 28 AFLP primer combinations (PCs) which generated 170 polymorphic bands. About 69% (117) of the markers showed segregation distortion in the DH population which was heavily skewed (p<0.001) towards the male parental line (89 bands from 67/3 parent and 28 from 305E40 parent). On the contrary, in the F2 progeny only 11 markers (6%) were distorted, and thus was chosen as mapping population.

A total of 73 AFLP PCs (40 EcoRI/TaqI, 11 EcoRI/MseI, 11 PstI/TaqI and 11 PstI/MseI) together with two microsatellites and a co-dominant marker linked to Rfo-sa1, a locus conferring resistance to Fusarium oxysporum, were applied and generated 405 polymorphic markers. By using the Joinmap software we identified 12 major (5 or more markers) and 2 minor (triplet and doublet) Linkage groups (LGs); 348 markers (86%) were assigned to these LGs while 56 markers remained unlinked (LOD ≥ 4).

A total of 204 markers were ordered to constitute the framework map which spanned 580.2 cM, with an average map distance of 4.2 cM. The length of major LGs ranged from 79 cM (LG1) to 18 cM (LG12). Surprisingly a marked clusterization was detected in LG1, which included 44 ordered markers together with further 120 accessory markers.

At present a wide set of SNP and other markers is under development and it is expected to stoutly contribute in saturating the eggplant molecular map for future QTL analyses of key agronomic and resistance traits.
PHOSPHOLIPID FATTY ACIDS IN ANTHERS OF EGGPLANT (S. MELONGENA L.), ALLIED SPECIES AND CORRESPONDENT INTROGRESSION LINES


*) Dipartimento di Ingegneria e Tecnologie Agro-Forestali, Facoltà di Agraria, Università degli Studi di Palermo, Viale delle Scienze 13, (Edificio 4), 90128 Palermo, Italy
**) CRA-ORL, Unità di Ricerca per l’Oriolcultura, Montanaso Lombardo (LO), Italy

Solanum aethiopicum, S. integrifolium, S. sodomaeum, Interspecific hybridization, PLFA

Lipids are structural components of the plant cells. In male gamete, have been also reported that lipids are precursors of signalling molecules involved in anther development, in pollen-stigma interaction, in flower attraction towards pollinators. Moreover, a possible role of fatty acids in triggering defence reactions to verticillium wilt in eggplant was also proposed.

Phospholipid fatty acids (PLFAs) analysis was carried out to characterize eleven eggplant genotypes (of which three employed as recurrent parents) and its allied species S. aethiopicum gr. gilo, S. integrifolium and S. sodomaeum employed in an introgression breeding program based on sexual and somatic hybridization. Thirty-one advanced introgression lines obtained after several cycles of backcrosses and selfing and four selfed progenies from the somatic hybrids were analyzed as well. Gas chromatography and mass spectral analysis were employed to identify and quantify the PLFAs, including palmitoleic, oleic, linoleic, linolenic, etc. Preliminary analyses demonstrate that the whole anther containing mature pollen gives comparable results to those from isolated pollen grains. Therefore, the biochemical analyses were carried out in triplicate using whole anther from open field grown plants. PLFAs analysis results were subjected to principal component analysis (PCA). The total amount of lipids (mg g⁻¹ fresh weight) varied greatly among all the genotypes tested, ranging from 16.8 to 0.3 respectively in a recurrent eggplant line and in a introgression line from S. aethiopicum. The most abundant fatty acids were 16:0 and 18:2w9,12. In the three allied species, the 12:0 (lauric acid) and 20:1w9 (gadoleic acid) fatty acids were not detected, whereas the 16:1w9 was absent in the recurrent eggplant genotypes. These fatty acids represented a minority fraction of the lipids. Interestingly, the 12:0, 20:1w9 and/or 16:1w9 were again detected at trace level in some introgression lines. This fact may indicate that, likely, a metabolic complementation occurred, enabling some gentopyes to synthesize these minor fatty acids in the anthers.

PCA identified three principal components explaining 83% of the observed variance. The PC1, which accounts for 64% of variance, showed the higher loadings for 16:0, 18:0, 18:1w9; 18:2w9,12, 20:0, 18:3w9,12,15, 20:1w9, 22:0 fatty acids and for the total lipids content. The PC2 accounts for 10% of variance and showed a positive loading for 16:1w9 and a negative one for 12:0.

The PC1 allowed to discriminate the different solanaceous species and sub-groups within the introgression lines analyzed. The PC2 was able to discriminate only two recurrent eggplants, S. aethiopicum, S. integrifolium and one introgression lines which showed the presence of all PLFAs. In conclusion, PLFAs analyses in anther may represent a further tool for a finer characterization of eggplant genotypes.
COMPARATIVE STRUCTURAL GENOMICS BETWEEN INCORRUENT WILD POTATO SPECIES


*) University of Naples Federico II, Department of Soil, Plant, Environmental and Animal Production Sciences, Via Università 100, 80055 Portici, Italy
**) University of Minnesota, Department of Plant Pathology, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108 USA

Wild potato species are an important source of disease resistance genes. Due to sexual incompatibilities with cultivated potato, some of these genes are unavailable for potato improvement. Sequence informations, structural genomics and biotechnology are changing this scenario. To improve access to the wild potato gene pool we have developed linkage maps for two wild potato relatives, *Solanum bulbocastanum* and *S. commersonii* using Diversity Array Technology (DArT). These species represent phylogenetically distinct series within the potato tertiary genepool. Here we report details and comparisons of generated linkage maps. To build the map of each species we used F1 mapping populations. Seven hundred fifty and 1000 mappable segregating DArT markers of *S. bulbocastanum* and *S. commersonii* have been found, respectively. About 40 common markers have been identified. At LOD score 3, *S. commersonii* map consisted of 13 linkage groups whereas 14 linkage groups were identified for the *S. bulbocastanum* map. Length of *S. commersonii* map was higher than *S. bulbocastanum*. Resulting maps can be further augmented with other marker sources and this will be the first medium density genome-wide linkage maps for these species. A preliminary bioinformatics study of marker sequences revealed that most of the DArT markers originate from expressed regions. With the ongoing tomato and the cultivated potato genome sequencing projects, sequencing of DArT markers mapped on wild potato will allow comparison of genome-wide structure throughout the genus *Solanum.*
GENETIC TRACEABILITY OF PLANT VARIETIES BELONGING TO SPECIES OF AGRI-FOOD INTEREST BY MEANS OF DNA BARCODING: A CASE STUDY

NICOLÉ S.*, AMBROSI D.*, BELLUCCI E.**, LUCCHIN M.*, BARCACCIA G.*

*) Department of Environmental Agronomy and Crop Science, University of Padova, Viale Università 16 – Campus of Agripolis, 35020 Legnaro (Padova)
**) Department of Environmental Sciences and Crop Production, University of Ancona, Via Brecce Bianche – Monte d’Ago, 60131 Ancona

**common bean, cpDNA, ITS, DNA barcoding, SNPs**

DNA barcoding is a technique for identifying species by obtaining a short DNA sequence from a known gene and comparing it with databases of orthologous sequences from species of established identity. Our study deals with the use of DNA barcoding as a new tool to recognize different Phaseolus species and to assess genetic distinctiveness of P. vulgaris varieties. It is largely accepted that the mitochondrial gene COI can be considered the core of the global biodentification system for animals, while for the plant kingdom the research of the barcoding markers is slowing down by the difficulty of finding the gene analogous to COI. Currently, the most promising markers for plants are from the chloroplast genome because it owns the same attributes of the mitochondrial one: it is an unparentally inherited, non recombining and structurally stable genome. This project deals with the study of the potentials of DNA barcoding applied to several pure lines of Phaseolus vulgaris species belonging to wild, domesticated and cultivated common beans by means of multilocus approach that consisted in amplifying and sequencing plastid genic regions (rbcL, trnL and matK) and intergenic spacers (rpoB-trnC, atpB-rbcL, trnT-trnL and psbA-trnH) along with the nuclear internal transcribed spacers (ITS1 and ITS2). In particular several Italian pure lines and Mesoamerican and Andean landraces were arbitrarily selected as representative of gene pools on the basis of morphological seed traits and plant descriptors, along with a few P. coccineus, P. lunatus and Vigna unguiculata accessions adopted as reference standards and out-types. Our main goals were i) to test how different markers perform as DNA barcodes, mainly below the level of species; ii) to investigate the differentiation among varieties and how we can use barcode data to reconstruct where modern "Italian" varieties come from; iii) to evaluate how well different methods (tree based versus character based) help us answer the previous questions. Among all the sequences tested, the best performance as barcoders at varietal level was attributable to trnH-psbA intergenic spacer and trnL intron, while the other regions provided few point mutations. Regarding the method, the phenetic approach confirmed to be a powerful technique to correctly separate different species and to cluster accessions corresponding to members of the same species, while at varietal level DNA barcoding standard tree-building method revealed to be scarcely informative to discriminate gene pools and to identify varieties within P. vulgaris. Thus a second approach, the character-based system, was tested and it revealed to be useful to detect within P. vulgaris species a total of 16 haplotypes over all cpDNA regions corresponding to as many subgroups, each one made up by Mesoamerican or Andean accessions along with Italian accessions that clustered with one or the other gene pool. An important finding is that haplotypes of most domesticated and cultivated
accessions were clustered in tight sub-groups, with the exception of few haplotypes shared only by wild and ancestral accessions, irrespectively of their Mesoamerican or Andean gene pool of origin. In conclusion, the DNA barcoding confirmed to be a very powerful technique to distinguish different plant species, but revealed to be poorly informative for the genetic traceability of single plant varieties.
MORPHOLOGICAL AND NUCLEOTIDE DIVERSITY IN WILD AND CULTIVATED COMMON BEAN (*PHASEOLUS VULGARIS* L.) ACCESSIONS FROM DIFFERENT GENEPOOLS

GIOIA T.*, LOGOZZO G.*, IERARDI G.*, ATTENE G.*, NEGRI V.**, SPAGNOLETTI ZEULI P.*

*) Dipartimento di Biologia Difesa e Biotecnologie Agro-Forestali, Università degli Studi di Basilicata, Viale dell’Ateneo Lucano 10, 85100 Potenza, Italy - tania.gioia@unibas.it.
**) Dipartimento di Scienze Agronomiche e Genetica Vegetale Agraria, Center for Biotechnology Development and Biodiversity Research, Università degli Studi di Sassari, Via De Nicola, 07100 Sassari, Italy
***) Dipartimento di Biologia Applicata, Università degli Studi di Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy

*Phaseolus vulgaris* L., SNPs, morphological traits, diversity

To identify and characterize the genes responsible for phenotypic variation is an essential goal for geneticists and breeders. This study examined the organisation of diversity for morphological and molecular markers in a sample of 101 inbred genotypes of *Phaseolus vulgaris* L and 5 genotypes of *P. coccineus* and *P. lunatus*, used as controls. The sample has been obtained to represent the evolutionary history of common: wild and domesticated forms from Mesoamerican and Andean gene pools were included. The 106 genotype include: 11 wild Mesoamerican genotypes; 28 domesticated Mesoamerican genotypes (from Mexico and Central America); 29 wild Andean genotypes; 33 domesticated Andean genotypes (from South America); 5 genotypes of *P. coccineus* and *P. lunatus*.

The differences among and within common bean accessions were initially studied using a set of 29 phenotypic descriptors (14 qualitative traits and 15 quantitative traits). All data were analysed by uni and multivariate statistical analysis.

Variation among nucleotides sequenced at 2 loci was also investigated. A total of 924 bp of aligned sequence for each genotype were analysed. Collectively, the sequences of all genotypes defined 17 haplotypes, Andean genotypes showed the highest number of haplotypes. The number of indel polymorphism was of 19. Nucleotide diversity ranged from 0,0024 π/bp to 0,047 π/bp. Negative Tajima D values was found in Mesoamerican wild genotypes (-1,6508).

No reduction of diversity was observed in domesticated germplasm compared to the wild genepool.
NUCLEOTIDE DIVERSITY IN WILD AND DOMESTICATED PHASEOLUS VULGARIS L. FROM MESOAMERICA


*) Sezione di Agronomia e Genetica Agraria, Dipartimento di Scienze Ambientali e delle Produzioni Vegetali (SAPROV), Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy
**) Dipartimento di Scienze Agronomiche e Genetica Vegetale Agraria, Università degli Studi di Sassari, Via E. De Nicola, 07100 Sassari, Italy

domestication, crop evolution, common bean, SNPs

The common bean (Phaseolus vulgaris) is a diploid (2n = 2x = 22), annual species that is predominantly self-pollinating and is the most important grain legume for direct human consumption. For P. vulgaris, many aspects of its molecular and phenotypic diversity, migration dynamics and population structure are well known. To date, in contrast, little information is available on the level and extent of its nucleotide diversity. The common bean was domesticated independently in Mesoamerica and in the Andes, and the largest diversity of its wild and domesticated forms is found in Mesoamerica, where a single domestication event is believed to have occurred. The main aims of the present study were to develop SNP markers and to identify genes and genomic regions that are related to the adaptive processes during domestication of P. vulgaris. We developed 30 primer combinations to amplify and sequence the orthologous counterparts of genes previously studied in wild and domesticated soybean. All of the primer combinations were used for a preliminary selection of 10 loci. A sample of 24 genotypes was developed to represent the wild and domesticated Mesoamerican populations (18), including six additional genotypes from the Andean and phaseolin I gene pools. Here, we present and discuss the results from the sequencing of 15 gene fragments (including five loci previously identified as potentially under selection during the domestication process in Mesoamerica).
TRANSFER OF USEFUL GENES FROM *PISUM SATIVUM* TO *PISUM ARVENSE* AND VICEVERSA

CHIARETTI D.*, STAMIGNA C.*, CHIARETTI E.**, IANNETTA M.*, BOZZINI A.***

*) ENEA Centro Ricerche Casaccia, S. Maria Galeria, Via Anguillarese 301, 00123 Roma
**) AGROSERVICE S.p.A., Loc. Rochetta, 62027 San Severino Marche
***) CRA, Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Via Nazionale 82, 00184 Roma

*field and common pea, breeding, domestication*

Farmers and breeders have accumulated in centuries of cultivation several useful genes in *Pisum sativum*, utilized both as a horticultural and feeding crop. Roveja (*Pisum sativum ssp arvense*) is practically the wild species, with small, round and dark seeds, of special flavour, domesticated only for the indehiscent pods.

The purpose of the present work consists in the introduction into the Roveja of other characters of domestication, through a program of crossing with modern cvs of peas, in order to get ideotypes that maintain the flavour characteristic of the seeds of the Roveja, but with more advanced morfo-physiological and yield characters, typical of the cultivated pea and at the same time, to get new lines of pea with better characters of rusticity etc. Crosses were made between 6 selections of Roveja, with indeterminate habit, red flowers and round, small seeds and 3 cvs of *P. sativum*, characterized by *aphila* habit, smaller plant size, green, rough seeds. The recombination results are presented, with the isolation of *aphila* and *superaphila* types, facilitating the cultivation and harvesting of Roveja and increasing the availability of some useful characters in *sativum*, including the description of some characters previously not present or uncommon both in *sativum* and *arvense* types.
GENETIC DIVERSITY OF ETHIOPIAN GRASS PEA (*LATHYRUS SATIVUS* L.) USING MOLECULAR MARKERS

SHIFERAW E.*, PONNAIAH M.*, PORCEDDU E.**

*) Scuola Superiore Sant’Anna, International Doctoral Programme on Agrobiodiversity- Plant Genetic Resources ENEA-Cr. Casaccia, Rome, Italy
**) DABAC, University of Tuscia, Via S. C. De Lellis, 01100 Viterbo, Italy

*Lathyrus sativus*, genetic diversity, EST-SSR

Grass pea (*Lathyrus sativus* L.) is a drought tolerant leguminous food crop and widely cultivated in Ethiopia, where in 2006/2007 covered an area of 124, 954 ha which is equivalent to 9% of total pulse growing area. Grass pea contains a non-protein amino acid called β-N-oxalyl-L-a, β-diaminopropionon acid (ODAP) which causes paralysis of lower limbs if consumed as a staple food for a long period of time. This contrasts to appealing properties such as its ability to grow in drought-striken, rain-fed areas with poor soil quality, its tolerance to extremely dry conditions and excessive floods. In areas stricken by frequent drought, grass pea becomes the only available source of food for the poor section of the population.

To date very limited molecular knowledge and molecular tools are available on grass pea in general and Ethiopian grass in particular. Here we present our research activity aimed at shedding light on the molecular diversity among the Ethiopian grass pea, by means of different sets of PCR-based markers. Diversity study was done on 320 genotypes of grass pea collected from different geographical regions of Ethiopia. Polymorphisms were detected using 21 Expressed sequence tagged (EST) derived Sequence Tagged (STS) markers chosen from the previously published data, and 19 EST-SSR markers newly designed by us from the publicly available *Lathyrus sativus* EST sequences. Out of the 21 STS markers 11 gave RAPD-like profiles, and 10 gave monomorphic bands which were converted to CAPS markers. From the total markers analysed 7 RAPD-like, 6 CAPS and 8 EST-SSRs showed polymorphism among and within accessions. Here we provide the first report on the pattern of genetic variation among and within the populations in Ethiopian grass pea and also the variation among different geographic regions. In addition we also tested the potential for across-species transferability of the 12 EST-SSR to other widely used legumes, such as chickpea, field pea, faba bean and lentil, demonstrating a high level of transferability. Thus this work is the first attempt to produce molecular tools to be utilized in germplasm characterization and in marker-assisted breeding in *Lathyrus* and related spp.
ASSESSMENT OF GENETIC RELATIONSHIPS WITHIN CUCURBITA PEPO USING RAPD AND AFLP MARKERS

FORMISANO G.*, PAPARO R.*, MANCUSO T.**, ERCOLANO M.R.*

*) Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples “Federico II”, Via Università 100, 80055 Portici, Italy
**) La Semiorto Sementi S.r.l, Via Vecchia Lavorate 91/93, 84080 Lavorate di Sarno, Italy

Cucurbita ssp., genetic distance, molecular markers, RAPD, AFLP

Cucurbita pepo, is among the economically most important vegetable crops worldwide and is grown in almost all temperate and subtropical regions. It is the most polymorphic species in the plant Kingdom, exhibiting the widest fruit characteristics variation. Most of fruit variability among cultivated C. pepo can be attributed to the different quality characteristics of the mature fruit flesh. Botanical classification based on allozyme variation recognized three subspecies. For an effective breeding program a knowledge of extent and nature of genetic diversity within and among subspecies is an important prerequisite. Genetic information will be useful for organizing germplasm collections, to characterize individual accessions and genotypes, to identify cultivars as well as a general guide in parents selection for hybridization to maximize of heterosis.

In recent years molecular markers were considered a powerful tool in the assessment of genetic diversity within and between plant population. The goal of this study was to identify the polymorphisms and to assess relationships between 17 genotypes which belong to C. pepo subsp. pepo. All genotypes exhibited morphological variation in some characters such as fruit shape, fruit size and fruit color. Two PCR molecular markers were employed: AFLP markers (amplified fragment length polymorphism) and RAPD markers (random amplified polymorphic DNA). In particularly, five EcoRI/MseI AFLP primers produced 644 bands, some of which were polymorphic. Genetic distances were estimated and a dendrogram using a UPGMA method was obtained. Genotypes cluster in three main groups. The highest similarity index (88.0%) was observed between the 2Z and 4Z genotypes. The analysis of the data RAPD is in progress.
A SEARCH FOR MOLECULAR MARKERS LINKED TO APHID RESISTANCE GENES IN LETTUCE BASED ON NBS-LRR DOMAINS

BERETTA M.*, BAGNARESI P.**, SABATINI E.*

*) CRA-ORL Unità di Ricerca per l'Oroicoltura, Via Paullese 28, 26836 Montanaso L. (LO)
**) CRA-GPG-Centro di Genomica e Post-Genomica Animale e Vegetale, Via S. Protaso 302, Fiorenzuola d'Arda (PC)

resistance gene, plant disease, Lactuca sativa, Lactuca virosa, aphids

Aphids represents one of the most devastating pests in lettuce. Genetic resistance towards more than one species of aphids was found in wild relatives of *Lactuca sativa*; *L. virosa* is one of the most promising sources of resistance.

ESTs mining based on specific primers for NBS-LRR as well as further related domains (based on ProDom website,; http://prodom.prabi.fr ) was performed on *L. sativa* and *L. virosa* ESTs databases. Towards this end, a variety of approaches were tested using R language (http://www.r-project.org/; http://www.bioconductor.org/). In particular, several versatile and memory-efficient string searching/alignment tools as offered in the “Biostrings” package were employed in order to identify as yet unannotated putative NBS-LRR genes.

Furthermore, primers specific for genes containing NBS-LRR domains and related to aphid resistance in other species were used in a modified Bulk Segregant Analysis approach, on populations of *L. sativa* segregant for aphids resistance.
MOLECULAR MARKER-ASSISTED INTROGRESSION OF WILD ASPARAGUS SPECIES GENOME INTO THE CULTIVATED ASPARAGUS OFFICINALIS

VALENTE M.T., SABATINI E., CASALI P.E., FALAVIGNA A.

C.R.A. – ORL Unità di Ricerca per l’Orticoltura, Via Paullese 28, 26836 Montanaso Lombardo (LO) (Italy)

Asparagus officinalis, interspecific hybridization, di-haploid, molecular markers

Interspecific hybridization in Asparagus Genus is expected to introgress desirable characteristics of wild species (good taste of spears, adaptation to biotic and abiotic stresses) into the cultivated one (A. officinalis L). To this purpose two species spontaneous in the Mediterranean climate were considered: A. maritimus L., resistant to rust disease (Puccinia asparagi) and tolerant to salt; A. acutifolius, adapted to xerophytic conditions. Besides both species are well known for good taste of their spears. To overcome the sexual incompatibility between A. officinalis and A. acutifolius a bridge-cross “A. officinalis (4n) x A. maritimus (4n)” was necessary. The “three-ways” interspecific hybrid plant obtained (OMA) was crossed to the tetraploide A. officinalis cultivar Violetto d’Albenga and eight BC1 (OMAO) plants were generated. One of these plants gave nine dihaploid (DH) and seven tetraploid androgenetic clones following in vitro anther culture. Dihaploid plants crossed with diploid A. officinalis genotypes gave F1 fertile plants (DO).

The objective of this study was the assessment of genomic introgression of A. acutifolius and A. maritimus into progenies derived from backcross to A. officinalis by means of molecular markers. To achieve this aim twenty-three RAPD markers were chosen for their high polymorphisms rate. Out of seventy species-specific polymorphic bands shown, fifty-eight (83%) were A. acutifolius-specific, while the remaining 17% could not be clearly attributed to A. maritimus or to A. officinalis. Such results confirm that these two species are genetically strongly related and distant to A. acutifolius.

All twenty-three RAPD markers were used to analyse OMA, OMAO, DH and DO plants. Results obtained showed that A. acutifolius genome was present in the OMA plant, but most of it was lost in the genotypes derived both from backcrosses with A. officinalis and anther culture. Moreover, the genome introgression of A. maritimus was not demonstrated because none of marker used discriminated this species from A. officinalis).

The molecular results found met with morphological observations. The interspecific hybrid OMA clearly showed some traits of A. acutifolius parent, while the progeny derived from backcross to A. officinalis (OMAO) did not. On the other hand it was possible clearly detect the recombination of many phenotypic markers associated to A. officinalis and A. maritimus: anthocyanic color, size and bitter taste of spear, occurrence of stipule at basis of stalk branching, bushy habitus, colour and size of the berry.

Research works are in progress to characterize advanced backcrossed progenies derived from interspecific hybridization for resistance to rust and Stemphilium diseases and other usefull traits of the two wild species. Considering the long time requested to breed asparagus (dioecious and perennial species), we are also looking for molecular markers associated to these traits for MAS.
GLOBE ARTICHOKE DESCRIPTORS AND THEIR IMPROVEMENT

REY N.*, PAGNOTTA M.A.*, NOORANI A.*, CRINÒ P.**, TAVAZZA R.**, ALERCIA A.***, SACCARDO F.*

*) Università della Tuscia, Via S. C. de Lellis, 01100 Viterbo, Italy
**) ENEA C.R. Casaccia, Dipartimento Biotecnologie, Agroindustria e Protezione della Salute, Via Anguillarese 301, 00123 Rome, Italy
***) Bioversity International, Via dei Tre Denari, 472/a, 00057 Rome, Italy

UPOV, descriptors, Cynara, stress tolerance, chitinase

The International Union for the Protection of New Varieties of Plants (UPOV) has developed a list of 51 descriptors of globe artichoke. This has now been adopted by the EU as the “protocol for distinctness, uniformity and stability (DUS) tests in Cynara scolymus (Cynara cardunculus var. scolymus L.)”. The descriptors are based on morphological traits, the recording and analysis of which is required for varietal evaluation and registration. The final version of the UPOV descriptors is dated 25/03/2004 (TG/184/3 Globe Artichoke, 2001-04-04). Of the 51 morphological traits, four characteristics may be used for grouping the accessions: (a) Leaf: incisions (10 to 12 leaf stage) (characteristic 9); (b) Central flower head: shape in longitudinal section (characteristic 26); (c) Central flower head: time of appearance (characteristic 28); and (d) Outer bract: colour (external side) (characteristic 41). These traits are useful for the description of artichoke accessions; assessment of some of the reported traits is however time-consuming, without providing essential information, and/or are highly affected by environment. Moreover, certain traits are ambiguous and open to interpretation, and unclear as to the stage and organ where the character should be recorded. Hence, in the present paper we propose fine-tuning some of the UPOV descriptors.

In our previous work on the Romanesco artichoke, high correlation was found among some of the morphological traits, showing a possible reduction in the number of descriptors without affecting the efficient discrimination among the Romanesco clones analysed. Results of Principal Component Analysis linked the following descriptors: (i) number of primary and secondary heads, total production, total weight of the secondary heads, and number of first order heads; (ii) weight of the central head, diameter of the central head, length of central head, main stem length, plant height, main stem diameter and time of appearance of the central head; and (iii) diameter of the first flower head on the lateral shoots, length of the first flower head on the lateral shoots and weight of the primary heads.

In the present work, run under the Action 036 CYNARES funded by European Commission, Directorate-General for Agriculture and Rural Development, under Council Regulation (EC) No 870/2004, we validate the data and we suggest a reduced list of descriptors to be adopted in globe artichoke. Moreover, we propose a possible standard leaf to be identified in order to record a series of traits.
EPIGENETIC CONTROL OF DEVELOPMENT IN AXENIC CULTURE OF GLOBE ARTICHOKE

de VIRGILIO M.*, TAGARELLI A.**, MORONE FORTUNATO I.**, FINETTI-SIALER M.*, PIGNONE D.*

*) Institute of Plant Genetics – CNR, Via Amendola 165/A, 70126 Bari
**) Department of Science of Vegetable Production, University of Bari, Italy

epigentic, development, artichoke, in vitro culture

In vitro propagation of globe artichoke is widely applied for large scale production. It is well known that in vitro propagation can induce epigenetic modifications responsible for different gene expression patterns and consequently altered phenotypes.

To establish a possible correlation between altered phenotypes and epigenetic modifications at DNA level, artichoke plants were grown in vitro in the absence or presence of 5-azacytidine, a DNA-methylation inhibitor.

Six subcultures were performed. At each step morphological traits of treated and untreated plants were recorded and genomic DNA was extracted from a number of samples.

Preliminary observations suggest a reduction in overall DNA-methylation level in plants treated with 5-azacytidine. Variations in plant development will also be described.