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Oral Communication Abstract – 6A.01

GENOMIC APPROACHES TO DISSECT THE GENETIC BASES OF STRESS TOLERANCE IN CEREALS

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Drought tolerance, Marker-assisted selection, QTL mapping, TILLING

A large number of genomic-based strategies and tools are currently available to dissect the highly complex genetic basis of stress tolerance in plants, with the final aim of improving crops. On one side, high-throughput functional genomics are contributing to identify the main key loci and the network of physiological processes involved in stress perception, signal transduction and response at the cellular, organ and plant levels. On the other side, QTL mapping and the description of allelic and haplotypic diversity are moving at unprecedented speed to depict the genetic bases of stress tolerance in crops germplasm, in order to identify more desirable alleles. In the process to translate this knowledge to breeding, complicating factors are the co-occurrence of multiple stresses and the difficulty to properly phenotype the high number of plants usually required in order to tailor novel cultivars more resilient to abiotic stresses. Among these, drought is the most recalcitrant and difficult to work with, also due to its unpredictability. We will describe a number of examples from our work on drought-related traits in durum wheat, barley and maize where genomics has significantly contributed to acquire useful knowledge which could be translated in breeding applications by means of marker-assisted selection and genetic engineering.

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Oral Communication Abstract – 6A.02

EARLY AND LATE EVENTS IN PLANT-HERBIVORE INTERACTIONS

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Successful defence depends on the plant's ability to recognize an attacking "enemy" as early as possible. Early defence responses require enemy-initiated signalling cascades. The activation of specific responses requires recognition and appropriate response towards the attacking enemy and most of the events which finally lead to gene activation (the signalling pathway) occur within a few minutes. Damage-induced ion imbalances and modulations of channel activities are the first events occurring in the plasma membrane (PM) and result in rapid perturbations of the PM potential (Vm) involving variations of cytosolic Ca^{2+} concentrations and the production of reactive oxygen species (ROS), such as H_2O_2 and NO. The sophisticated signalling network for plant defence responses is elicited and driven by both herbivore-induced factors (e.g., elicitors, effectors, and wounding) and plant signalling (e.g., phytohormone and plant volatiles) in response to arthropod factors. Furthermore, the ability of plants to withstand herbivores relies on direct and indirect chemical defence. By using toxic phytochemicals, plants can deter and/or poison herbivores, while by releasing volatile organic compounds (VOCs) into the atmosphere plants can attract predators of the herbivores. Interacting downstream networks of kinases and phytohormones mediate the signal and result in concerted gene activation. Here I review and discuss early and late events occurring during herbivore attack that are responsible for cascades of events and signal transductions, eventually leading to indirect and direct plant responses.

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Oral Communication Abstract – 6A.03

IDENTIFICATION AND CHARACTERIZATION OF INDUCED MUTATIONS IN A SUNFLOWER TILLING PLATFORM

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Helianthus annus, reverse genetic, candidate genes

The TILLING strategy has been successfully applied to the sunflower genome in our laboratory ("sunTILL platform"). The interest was first focused on some key enzymes of the fatty acid pathway, because of the interest in increasing the nutritional value of sunflower oil by the reduction of the ratio of saturated to unsaturated fatty acids. Moreover, *P. halstedii* is one of the most dangerous pathogens that affects sunflower cultivation in the Mediterranean area. Therefore the availability of a stable and effective system, as genetic resistance, for the pest-control results of prime importance. Thereby, a pilot assay on 1,152 sunflower M_2 lines was carried out by the reverse genetic screening of four genes: the *kasII* and *kasIII* genes, respectively codifying the isoforms II and III of the β-keto-acyl-ACP-synthetase; the *fad2-1* gene, encoding the enzyme responsible of the converting reaction of oleic acid to linoleic acid; the *AY490791* gene, involved in *P. halstedii* resistance.

Since few genomic sequences are publicly available for sunflower, the reverse genetic screening was preceded by an accurate reconstruction of candidate gene models, by the amplification and the subsequent sequencing of short overlapping fragments. For each candidate gene the most promising region for TILLING analysis was thereby identified. In this way, new primer pairs flanking this region were set on the intronic sequences, with the aim to improve the screening efficiency on the coding regions.

In the pilot assay, nine mutant lines have been totally identified. The four mutations scored in the *kasII* gene were homozygous; three of them were localized in introns, while one caused a G/T transversion, resulting in a premature stop-codon (E139*). No mutant lines were identified for the *kasIII* gene. In the case of *fad2-1* gene, three mutations were identified: one resulted in a missense change (F26L), a second caused a silent change (R46=) and a third was situated in the non-coding region. The *AY490791* gene screening revealed two mutations, both localized in non-coding sequence. Each mutant line was then confirmed by sequencing and genotyped by microsatellite markers to exclude any individuals originating from cross-pollination events. The results of this first reverse genetic screening translated into an average mutation frequency of 1/475 kb.

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Oral Communication Abstract – 6A.04

DISCOVERY OF NUCLEAR MALE-STERILITY IN RED CHICORY: GENETIC ANALYSIS AND METHODS FOR THE MARKER-ASSISTED BREEDING OF F1 HYBRID VARIETIES

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Cichorium intybus L., male sterile mutants, F1 hybrids, crop productivity

The present research deals with the discovery and genetic analysis of male sterile mutants of red chicory (*Cichorium intybus*, $2n=2x=18$). Four distinct mutants[1], which to the best of our knowledge are the first spontaneous male sterile mutants ever discovered and described in the genus *Cichorium*, were characterized in great details for the developmental pathway of micro-sporogenesis and gametogenesis, and the inheritance pattern of the gene, here named *Cims-1*, underlying the male-sterility trait. A quick molecular diagnostic assay was also developed for the early marker-assisted selection of the genotype associated to male sterile plants. Overall data clearly support a nuclear origin and a monogenic control of recessive type for the male-sterility trait in each of the red chicory mutants. Male gametogenesis was documented to arrest at the stage of uninucleate microspores. In particular, cytological observations revealed that microspores degenerate before their release from the tetrads, later showing a collapse of the exine. In the mutants, the totality of microspores proved to be shrunken and much smaller than wild-type ones. In fact pollen grains were never detected in mature anthers, demonstrating a full expressivity of the trait with mutants being 100% male sterile. Moreover, the fine mapping of the mutant locus was attempted by molecular markers using F_2 and BC_1 populations segregating for male-sterility. The gene responsible for male-sterility was found tightly linked to a microsatellite of the TC/GA type whose full sequence was recently deposited in the NCBI databases under the accession no. JF748831. A molecular diagnostic assay was then developed to be profitably adopted as a tool of marker-assisted breeding and exploited for an early screening of male-sterile plants within segregating progenies stemmed from back-crosses, with a genotyping error lower than 3%. Four new hybrid varieties of radicchio *Rosso di Chioggia* with different earliness, spanning from 80 to 110 days, were bred during 2011 by crossing male sterile partially inbred clones, used as seed parents, with wild type highly inbred lines, used as pollen donors. The parental lines to be used in large-scale pair-wise crosses were selected on the basis of their specific combining ability (SCA) assessed by means of molecular marker analysis (*i.e.* genetic distances) and agronomic progeny tests (*i.e.* field performances). Hybridization between parental genotypes so chosen produced vigorous and uniform F_1 hybrids, presenting detectable effects of heterosis. On the whole, the constitution of F_1 hybrids seems profitable in a practical breeding scheme and it is also feasible on a large commercial scale by the selection of self-compatible genotypes, for the production of inbred lines, and the identification of male sterile genotypes, to be used as seed parents for the hybridization with unrelated pollen donors. The discovery of non-engineered male-sterility in red

chicory will open new frontiers for maximizing crop productivity in this important cultivated vegetable species through the breeding of heterotic F_1 hybrid varieties.

[1]PENDING PATENT APPLICATION NO. PCT/EP2011/058765

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Oral Communication Abstract – 6A.05

THE PRESSURE COLLAR TECHNIQUE APPLIED TO GRAPEVINE SHOOTS ELUCIDATES CONTRIBUTION OF ABSCISIC ACID (ABA) AND GENE EXPRESSION OF VESSELS ASSOCIATED CELLS (VACs) DURING EMBOLISM FORMATION AND REPAIR

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Aquaporins, drought, Vitis vinifera L., Laser Micro Dissection (LMD)

In woody plants, xylem embolization and ABA root-to-shoot signaling act together to trigger stomata closure.

The pressure collar (PC) technique was applied to shoots of grapevines to induce embolism formation (by PC pressurization) and recovery (by PC depressurization) in xylem vessels without metabolic interference. In parallel, embolism formation and recovery were induced on other plants by water stress (WS) and rehydration. The PC was applied on a branch of an irrigated plant, whereas another branch was used as irrigated (IRR) control. We measured leaf gas exchange and stomatal conductance (gs) with an Infra Red Gas Analyzer, leaf water potential (ψ_{leaf}) by pressure chamber technique, and hydraulic resistance by High Pressure Flow Metering (HPFM) to assess the percent loss of conductivity (PLC) caused by embolism. In addition, we assessed foliar ABA content.

Five hours of PC treatment increased PLC and reduced ψ_{leaf} and gs to values similar to WS plants. Thereafter, we depressurized PC branches and irrigated WS plants. In PC branches, ψ_{leaf}, gs and PLC recovered four hours later, whereas WS plants recovered after rehydration more slowly (two days later) following a gradual, ABA-affected recovery of gs.

Leaf petioles of plants subjected to PC or WS were sampled at end treatment (maximum stress conditions) contemporarily with petioles of IRR controls. In addition, PC and WS leaf petioles were sampled when PLC was recovered. The sampled petioles were processed in order to isolate Vessels Associated Cells (VACs) with Laser Micro Dissection (LMD) technique after paraffin embedding. To analyze VACs activity during the cycles of formation and recovery of embolism, transcripts of sugar transporters, genes related to ABA metabolism, aquaporins and stress-related transcription factors were detected by RT-PCR assays. VACs metabolic involvement, as specifically triggered during embolism formation and recovery, was elucidated and discriminated from recovery from water stress.

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Oral Communication Abstract – 6A.45

ERF16, A JA-INDUCED AP2/ERF TRANSCRIPTION FACTOR OF *ARABIDOPSIS THALIANA*

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Methyl jasmonate, wounding stress, Arabidopsis thaliana, ERF (Ethylene-Responsive Factor)

We identified *ERF16* (Ethylene-Responsive Factor 16) as a jasmonate- and wounding-inducible gene. *ERF16* encodes a member of the A-5 subfamily (group II) of AP2/ERF transcription factor family, whose function has not been investigated so far. In wild-type (Col-0) plants, the gene was predominantly expressed in roots and flower stems but poorly expressed in leaves. Two *ERF16* homologs*, ORA47* and *At1g19210,* displayed similar expression patterns in different Arabidopsis organs, in response to abiotic stress conditions and in the presence of exogenous phytohormones. We placed the three Group II ERFs along the jasmonate signaling pathway using the Arabidopsis *coi1-16* mutant. Only *ERF16* was found to rely on COI1 for induction by jasmonates, indicating that the three Group II ERFs do not all participate to the same signaling pathway and only *ERF16* can be placed in the COI1-dependent pathway. A T-DNA insertion in the genomic region upstream of *ERF16* caused up-regulation of the gene itself and other MeJA-responsive genes confirming that *ERF16* plays a role in the jasmonate signaling pathway.

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Poster Communication Abstract – 6A.07

MAPPING QTLs FOR ROOT MORPHOLOGICAL TRAITS IN DURUM WHEAT

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Durum wheat, root morphology, recombinant inbred lines

Rooting depth is among the most important traits required to sustain plant function under low water availability conditions. A deep and voluminous root system should permit seedlings to extract soil moisture in a greater soil volume. The information available on the genetic control of root traits in the field and their relationships with yield is limited, mainly due to the difficulty of measuring root characteristics in a large number of plants. The introduction of DNA-based molecular markers, allows for unprecedented opportunities to identify the genetic factors underpinning the variation of quantitative traits and to investigate to what extent linkage and/or pleiotropy may influence traits association. This work represents a first attempt to study chromosome regions involved in the control of root architecture by testing a recombinant inbred lines (RIL) population derived from the cross between two durum wheat varieties (Creso and Pedroso) contrasting for root traits. The genetic map comprised more than 500 molecular markers spanning greater than 1800 cM. QTL analysis showed that a relatively limited number of chromosome regions were involved in the root morphology. The most relevant regions were identified on chromosome 2A, 6A, 5A and 1B for traits related to length, area and volume of roots. The mapping of the QTLs of root morphological traits in durum wheat should facilitate breeding for drought resistance.

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Poster Communication Abstract – 6A.08

THE SECRETORY PHOSPHOLIPASE A_2 ($sPLA_2$) GENE FAMILY IN DURUM WHEAT: IDENTIFICATION, CHARACTERIZATION AND EVIDENCE FOR A ROLE IN ADAPTATION TO DROUGHT STRESS

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Triticum durum Desf., phospholipase A_2, drought stress

To survive adverse stresses plants have developed complex signalling networks to perceive environmental stimuli and transduce the information over the plasma membrane into the cell where it activates specific signalling cascade. In this context, the production of lipid mediators triggered by phospholipases, throughout the generation of membrane phospholipid-derived second messengers, plays a pivotal role in plant response to environmental stresses. PLA_2 specifically hydrolyses phospholipids at the *sn*-2 position to yield free fatty acids (FFAs) and lysophospholipids, which are known to act as signalling molecules in a wide range of physiological and pathological processes. Currently, the PLA_2 superfamily consists of five different classes: the secretory PLA_2s ($sPLA_2$), the cytosolic Ca^{2+}-dependent PLA_2s ($cPLA_2$), the cytosolic Ca^{2+}-independent PLA_2s ($iPLA_2$), the platelet activating factor acylhydrolases and the lysosomal PLA_2s. In plants, to the best of our knowledge, to this day, the only specific PLA_2s discovered belong to the class of secretory PLA_2s.

In the light of this, an investigation was carried out to identify and characterize the $sPLA_2$ gene family in durum wheat and to evaluate its involvement in durum wheat response to drought stress. On the basis of sequence homology with the four $sPLA_2$ genes of rice (*OssPLA_2I*: Os02g0831700, *OssPLA_2II*: Os03g0261100, *OssPLA_2III*: Os03g0708000, *OssPLA_2IV*: Os11g0546600) the full-length sequences corresponding to the four durum wheat $sPLA_2$s genes and to their corresponding transcripts were isolated and characterised. The genomic structure of the four $sPLA_2$ genes was determined by comparisons between the gene sequences and the corresponding expressed sequences. The isoforms I comprised 3 exons and 2 introns, while the other three isoforms comprised 4 exons and 3 introns. While the length of the exons was highly conserved among the $sPLA_2$ genes, the introns showed a high degree of variability. The four expressed sequences were identical to the coding sequences deduced from the corresponding $sPLA_2$ genes, thus demonstrating that they are all actively transcribed and potentially encode functional $sPLA_2$s isoforms. Structure analysis of the deduced proteins showed that the putative proteins should be functional $sPLA_2$s isoforms as they contain all the domains typical of the plant $sPLA_2$s.

In leaves two genes encoding the isorform I and III were found to be up-regulated by water deficit, while the other two were found to be constitutively expressed. Consistently, as a consequence of the stress imposition, an increase was also observed in a Ca^{2+}-dependent PLA_2 activity whose biochemical characteristics resemble those of other well known plant $sPLA_2$s. In line with these findings, the analysis of the FFA pool of durum wheat leaves under water deficit revealed an increase in the amount of polyunsaturated molecules.

In the whole, the results obtained reveal the existence in durum wheat of a gene family encoding putative $sPLA_2$s and suggest a role of specific $sPLA_2$s isoforms in durum wheat response to water deficit.

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Poster Communication Abstract – 6A.09

INTRA-SPECIFIC MAP OF DURUM WHEAT BASED ON SSR MARKERS

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Recombinant Inbred Line, LOX, Simple Sequence Repeats, A/B-genome

Now a day several technologies are available to increase the abundance of DNA markers and to contribute in developing high resolution genetic maps suitable for genetic analysis. Among those simple sequence repeats (SSRs), highly dispersed in the genomes, were used for designing genetic maps and, in turn, estimating the genetic relationship between the A and B genome. A genetic linkage map of tetraploid wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.) will construct based on a intraspecific cross between two recombinant inbred lines (RILs): one deriving from the cross of Jennah Khetifa x ChamI, the other from the cross [Omrabi x Dicoccoides] x Omrabi.

The aim of this study is to expand the number of simple sequence repeat (SSR) and single-nucleotide polymorphism (SNP) markers on the wheat array that can be mapped in the wheat genome, and to determine their chromosomal location.

The 190 single-seed descent lines derived F8 RILs were analyzed with a total of 204 loci SSR and 20 loci SNP. After 7 generations of selfing looking at two genes in the F8, we will get a percentage of 98.45% homozygousity and 1.55% heterozygosity. It was also variable the number and location of SSR on chromosomes. The total number of markers and the density was higher in homoeologous groups 2 and 4 (with respect of a total of 73 and 35). Here are presented the ongoing results. After analyzing a total of 204 markers,, 113 markers SSR revealed polymorphism between parents, while the rest were monomorphic. These markers were previously located on chromosome arms in ditelosomic and nullitetrasomiche aneuploid lines of *T .aestivum* cv. Chinese Spring. The SSR markers were distributed on all chromosomes of the two genomes in durum wheat (A and B), with the highest percentage of polymorphism in the A-genome (64%) comparing with the B-genome (57%). It was also variable, the percentage of polymorphism, both between chromosomes and between genomes of the seven homologues in this of tetraploid wheat species, The homology groups 1, 4 and 6 in the A-genome showed a highest level of polymorphism (54%, 21%, 38%, respectively).

We are going to built a genetic linkage map. The total number of markers and its density is higher in homoeologous groups 2 and 4 (84 and 39 SSR). Each marker corresponds to one locus. We are also looking for specific molecular markers on the LOX and DREB genes, among some markers we have chosen and selection four markers (WMS251, WMS149, WMC349 and WMC47), because the literature shows that these markers were highly associated with genes that are interested. This map will provide useful groundwork for further genetic analyses of important quantitative traits, positional cloning, and marker-assisted selection, as well as for genome comparative genomics and genome organization studies in wheat and other cereals.

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Poster Communication Abstract – 6A.10

VALIDATION OF GRAIN PROTEIN CONTENT IN DURUM WHEAT

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Grain protein content, durum wheat, molecular markers

Marker assisted selection (MAS) is a powerful tool for traits like grain protein content (GPC) of durum wheat (*Triticum turgidum* L. convar *durum*), which exhibits high genotype-environment interaction leading to low heritability. In a previous work, undertaken to identify molecular markers associated with QTLs for GPC in durum wheat, Blanco et al. (2011) identified some QTLs in the RIL mapping population Svevo x Ciccio evaluated under different enviromental conditions. In order to employ these QTLs in breeding programs, additional studies are necessary to validate them in different genetic backgrounds where the GPC loci should be expressed without penalties on grain yield. A good way is to verify the effects of the QTLs in a different mapping population. An alternative way is to obtain near isogenic lines (NILs) for the two alleles of the target QTL using the markers identified for that QTL.

Three QTLs, localized on chromosome arms 1AL, 2AS, and 4AL, respectively, were validated in a recombinant inbred line population developed by crossing the durum wheat cultivars Svevo and Duilio and evaluated in two environments for three years. Selection for the positive allele resulted in 0.12 to 0.22% increase in GPC. The molecular markers *Xgwm95* and *Xgwm339*, significantly associated with the grain protein content, showed little effect on yield in most environments.

Six NILs sets, each consisting of two homozygous genotypes, were developed starting from heterozygous lines at the marker associated with the grain protein QTL on chromosome arms 1AL, 2AS and 3BS from the RIL Svevo x Ciccio. The phenotypic analysis were performed on two genotypes of each NIL grown in two environments for two years and evaluated for grain protein content and grain yield per spike. The QTL for GPC located on 2AS, associated with *Xgwm339*, was validated in different environmental conditions and was not correlated with yield per spike. The Svevo allele at this marker showed an additive effect on GPC (0.25 to 0.40%). Given the consistent expression pattern in multiple populations and environments, *Xgwm339* can be used for marker-assisted selection for high GPC.

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Poster Communication Abstract – 6A.11

SNP DEVELOPMENT AND VALIDATION IN DURUM WHEAT USING NEXT GENERATION SEQUENCING (NGS) TECHNIQUES

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Single nucleotide polymorphisms (SNP), Triticum durum Desf., Next Generation Sequencing (NGS), linkage mapping, Illumina assay

In durum wheat (*Triticum durum* Desf.), an allotetraploid species (AABB genomes), the development of SNP assays is complicated by the presence of two highly similar homeologous copies for each gene, ancestral paralogous and highly repetitive sequences. Collectively, the combination of low nucleotide diversity, polyploidy and repetitiveness severely limit advances in SNP discovery and genotyping platform development. We describe the application of Complexity Reduction of Polymorphic Sequences (CRoPS®) technology coupled with the Illumina Golden Gate (GG) genotyping assay and of Genotyping by Sequencing for SNP identification and genotyping in the elite durum wheat germplasm.

Starting from the genomic DNA of four diverse durum wheat genotypes (Neodur, Colosseo, Claudio and Rascon), SNP discovery was performed following the CRoPS protocol (van Orsouw et al. 2007. PLoS One 2:e1172) which included a preliminary step of genome complexity reduction based on the AFLP technique and a massively parallel sequencing of the libraries. The tagged libraries from the four genotypes were sequenced in one single run of GS FLX 454 Roche sequencer. A total of 2,659 SNPs were identified on 1,206 consensus sequences. Among the 768 SNPs that were randomly chosen irrespective of their genomic repetitiveness level to be assayed on the Illumina BeadExpress genotyping system, 275 (35.8%) SNPs passed the validation phase. Of these SNPs, 157 were mapped in the biparental mapping populations Colosseo x Lloyd (Mantovani et al. 2008. Mol Breed 22:629–648) or Meridiano x Claudio (Maccaferri et al. 2011. TAG DOI 10.1007/s00122-011-1605-9) for which SSR- and DArT-based framework maps were available. The Illumina genotyping assay of the RILs was carried out on pre-amplified templates to achieve the same level of genomic complexity reduction (*Pst*I + A/*Taq*I + CT) used during the SNP discovery phase. Considering the non-repetitive sequences only, the proportion of correctly genotyped SNPs increased to 47.0% (Trebbi D. et al. 2011. Theor Appl Genet DOI 10.1007/s00122-011-1607-7).

In a second NGS experiment, Genotyping by Sequencing (GBS; Elshire et al. 2011. PLoS One. 6:e19379) was used for the first time in *Triticum durum* to identify and genotype SNPs at the same time. Illumina highly parallel sequencing technology was used to sequence and genotype reduced representation libraries from 91 RILs of the Colosseo x Lloyd mapping population. The sequencing experiment was conducted with pools of 14 tagged genotypes per each Illumina sequencing flow-cell. This allowed to generate and map with high confidence ca. 1,000 high quality

SNPs with less than 10% overall missing data, i.e. a subset of all the SNP information generated with the Illumina sequencing experiment. Our study contributes towards a more cost-efficient and high-throughput whole-genome mapping in wheat.

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Poster Communication Abstract – 6A.12

FINE MAPPING OF THE LEAF RUST RESISTANT *Lr14* LOCUS IN DURUM WHEAT

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Leaf rust (Puccinia triticina Eriks.), durum wheat (Triticum durum Desf.), Lr14, synteny, association mapping

Leaf rust (*Puccinia triticina* Eriks. & Henn.) is a main disease affecting durum wheat production in the Mediterranean region. Resistance to this fungal pathogen is a main objective for durum wheat breeding. Improving the resistance to leaf rust can be effectively accomplished through mapping of the resistance loci from valuable sources and using marker-assisted selection (MAS) in breeding programs. The leaf rust resistant allele *Lr14-Creso* from durum wheat cv. Creso and its derivative Colosseo is one of the most important leaf rust resistance sources present in the modern durum wheat germplasm and it has been located in the distal portion of chr. 7BL (Maccaferri et al., 2008. TAG 117: 1225-1240; Marone et al., 2008. Mol Breed 24: 25-39). The identification of several closely linked SSR markers provides the necessary molecular tools to conduct MAS. Our target is to fine map *Lr14-Creso*. The RIL Colosseo x Lloyd population (176 lines) has been used to enrich the QTL region with new molecular markers derived from wheat ESTs. Phenotypic data were collected in the field and also at the seedling stage. A high heritability of the disease response was observed in both cases ($h^2 > 0.80$). The population allowed for mapping the locus at a good resolution level (5 cM).

A set of ca. 100 recombinant $BC_2F_{3:4}$ lines have been developed to fine mapping the QTL. Additional BC_3 lines have been developed in order to confirm and to further study the phenotypic effects of *Lr14-Creso***.** New SSRs and 13 EST-STS markers (UBW and MAG markers) were developed and mapped within an interval of 14 cM that includes the QTL peak.

The EST-STS markers have been obtained by exploiting the conserved colinearity between the most distal portions of rice chr. 6, *Brachypodium* chr. 1 and wheat chr. 7BL. Using the coding sequence of the rice and *Brachypodium* colinear genes, the corresponding wheat orthologs were retrieved using PpETS software. Specific PCR assays (ca. 1 kb) with the primers targeting the intron/exon boundaries of the genes were designed using Primer3, amplified on the genomic DNA of the parents Colosseo and Lloyd and the amplicons cloned in pGEM®-T Easy Vector. Eight clones for each parent were sequenced. Sequencing of the amplicons allowed for the identification of the SNPs differentiating the two homeologous copies of each gene (genome-specific SNPs) as well as the varietal-SNPs between Colosseo and Lloyd. These SNPs were then used to develop markers that, at the same time, were 7B specific and that were polymorphic between the two parents. The detailed synteny analysis and the map of the region including the newly developed markers will be reported. The results are supported by an independent association mapping study

carried out using a panel of 164 elite accessions (cultivars and advanced breeding lines). This allowed us to validate the presence of *Lr14* and to further improve the mapping resolution. The newly developed *UBW* markers tagging the *Lr14-Creso* allele are presently used in MAS activities.

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Poster Communication Abstract – 6A.13

MAPPING QTLS FOR GRAIN YIELD, YIELD COMPONENTS AND QUALITY IN DURUM WHEAT

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Durum wheat (Triticum durum Desf.), SSRs, DArT markers, QTL mapping, yield

A population of 180 $F_{6:7}$ recombinant inbred lines (RILs) developed at DISTA from the cross between the durum wheat cultivars Simeto and Levante was evaluated in four field experiments (Cadriano and Bologna in 2008; Argelato and Bologna in 2009) conducted according to a modified augmented design field layout. The following traits were considered: heading date (HD), grain yield (GY), thousand kernel weight (TKW), test weight (TW), grain yellow pigment content (GYPC), semolina color (SC), grain protein content (GPC) and gluten quality (SDS).

The population was genotyped with SSRs, DArT markers and PCR primers directly designed from known allelic variants of specific genes (UBW markers tagging *Lr14* and STS markers tagging *Psy* genes). The linkage map included 30 linkage groups, for a total length of 1.771 cM, containing 200 SSRs and 402 DArT markers. The map covers about 75-80% of the entire genome of durum wheat. HD was controlled by a major QTL (*QHd.ubo-2B*) expressed in both field experiments, with R^2 values of 24.5 and 27.2% in Cadriano and in Argelato, respectively, and by five minor QTLs with R^2 ranging from 5.3 to 15.1%, depending on the environment. The map location of the major QTL for HD is most probably coincident with that of the photoperiod-sensitivity locus *Ppd-B1*; however, further molecular analyses are needed to confirm this hypothesis. QTLs for GY were identified on chrs. 5A and 6A with R^2 values lower than 10%; the favourable alleles for GY were always contributed by Levante. A major QTL for TKW was identified on chr. 2BL (*Qtkw.ubo-2B*), distal side, with R^2 value up to 14.6% in Cadriano-2008 (between *gwm55* and *wmc546*). In this case, the favorable allele was conferred by Simeto. This QTL did not show concomitant effects on HD, GY, or other yield components and mapped ca. 40 cM proximal to *QHd.ubo-2B*. Four major QTLs controlled the grain yellow pigment content and semolina color; these QTLs, which were consistently expressed across the two locations, were located on chrs. 2BL (*QSc.ubo-2B*, R^2 ca. 8%), 5BL (*QSc.ubo-5B*, R^2 ca. 9%), 7BS (*QSc.ubo-7B.1*, R^2 ca.13%, in the proximal region) and 7BL (*QSc.ubo-7B.2*, R^2 ca. 6%, in the distal region). For all these four QTLs the favorable alleles were always conferred by the Levante. A major QTL on chr. 6A (*Qsds.ubo-6A,* with R^2 ca. 16%), was identified for gluten quality, as estimated with the SDS assay; in this case the favorable allele was conferred by Simeto. In general, both parents contributed favorable alleles for different traits, which allowed for the identification of transgressive RILs.

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Poster Communication Abstract – 6A.14

HIGH-RESOLUTION MAPPING OF A MAJOR QTL FOR GRAIN YIELD *PER SE* IN DURUM WHEAT

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Grain yield, durum wheat, major QTL, fine mapping, QTL cloning

In durum wheat, two major QTL for grain yield *per se* (*QYld.idw-2B* and *QYld.idw-3B*) and related traits were identified in a recombinant population derived from Kofa and Svevo (Maccaferri *et al*. 2008, Genetics). To further investigate the genetic and physiological basis of allelic variation for this important trait, the fine mapping of *QYld.idw-3B* is underway in the framework of the FP7 TriticeaeGenome project.

In this regard, 19 pairs of near-isogenic lines (NILs) for *QYld.idw-3B* were obtained from $F_{4:5}$ heterogeneous inbred families. In order to confirm the phenotypic effect of the QTL all pairs were evaluated in field trials in 2010 and 201. Three pairs of NILs, with contrasted haplotypes at the target region, were crossed to produce a large F_2 population (ca. 7,500 plants in total) that was screened with two flanking markers for the identification of recombinants. A total of 250 homozygous $F_{4:5}$ segmental isolines were obtained and the phenotypic and genotypic characterization of these materials is underway. To increase the map resolution in the interval of *QYld.idw-3B* new polymorphic markers were identified by exploiting the sequence information produced from the assembly of the chr. 3B physical map of bread wheat. Originally, *QYld.idw-3B* was mapped on the distal region of the short arm of chr. 3B, flanked by *Xgwm389* and *Xgwm493*. A total of 44 new markers (BAC-derived SSR, ISBP and SNP markers) have been added to the target interval, with an average marker distance of 0.28 cM. All markers were anchored to the Chinese Spring physical map of chr. 3B, which allowed us to identify the BAC Contigs spanning the QTL region and to assign the QTL peak to Contig 954, most probably between *Xcfb6127* and *Xcfb6021*. Sequencing of this contig has revealed the presence of 42 genes (Choulet *et al*. 2010).

Fine mapping will be carried out by genotyping the newly developed $F_{4:5}$ recombinant segmental isolines with all the available markers. The functional characterization of the genes included in Ctg954 is being carried out in a transcriptomic experiment with NIL pairs grown in the greenhouse that are being sampled for various tissues and developmental stages. This action will aim at identifying candidate genes based on the differential transcriptional patterns and will require the development of genome-specific assays for each gene.

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Poster Communication Abstract – 6A.15

THOUSAND KERNEL WEIGHT AND NUMBER OF STERILE SPIKELETS IN DURUM WHEAT: TWO SIDES OF THE SAME COIN?

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Thousand kernel weight, sterile spikelets, durum wheat, synteny, NGS

Association mapping was used to dissect the genetic basis of grain yield (GY) in a collection of 189 elite durum wheat accessions evaluated during 2004 and 2005 in 15 Mediterranean environments highly different for GY. Association mapping results evidenced the Colosseo-like haplotype as the favourable one for the main QTL for trait thousand kernel weight (TKW) on chr. 5A. (Maccaferri M. et al. 2011 Journal Experimental Botany. 62: 409-438). A population of 176 RILs from the cross Colosseo (C) x Lloyd (L) was used to obtain a linkage map based on 162 SSR and 392 DaRT markers for a total length of 2,022 cM. The CxL RIL population was evaluated for TKW in four field trials (2006 fall sowing, 2009 both fall and spring sowing, 2010 fall sowing). Moreover, in 2010 the CxL RIL population has been tested for several traits related to GY, including the number of sterile spikelets (St-Spk). Phenotypic data were analyzed by composite interval mapping (CIM) and QTLs were identified for both traits in the proximal portion of chr. 5AS (deletion bin 5AS1-0.40). Seven of the 120 SSRs tested between the parents of the mapping population, have been mapped in the 5AS region and the QTL analysis showed the presence of two QTLs for TKW and St-Spk with similar LOD profiles and QTL peak position. The region spans an interval of 12 cM. Therefore, there is the possibility that both TKW and St-Spk are under the genetic control of the same locus (pleiotropy). *QTkw.ubo-5A* may represent a locus controlling spikelet fertility during differentiation rather than a locus for grain weight *per se*. This needs to be confirmed with more detailed genetic materials and experiments. With regard to the genetic materials both BC_3 progenies and congenic lines derived from heterogeneous inbred families were obtained through MAS on both parental cvs. Colosseo and Lloyd.

Moreover, SNP markers from conserved orthologous regions (COS-SNP) were developed through sinteny-based analysis by exploiting the conserved colinearity between *O. sativa* chr. 12-*Brachypodium* chr. 4 and *O. sativa* chr. 9-*Brachypodium* chr. 4, respectively. SNP discovery between Colosseo and Lloyd showed low variability (*7%*) in the peri-centromeric region of chr. 5AS. Finally, we are using the "sequence capture" method in combination with "next generation sequencing" in the attempt to discover COS-SNP markers from at least 200 genes selected based on the collinearity with the target region.

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Poster Communication Abstract – 6A.16

ASSOCIATION MAPPING FOR RESISTANCE TO *SEPTORIA TRITICI* BLOTCH IN DURUM WHEAT

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Septoria tritici blotch (STB), Triticum durum Desf., association mapping, SSR markers, DArT markers

Durum wheat production in the Mediterranean basin is plagued by a range of biotic stresses. Among those, *Septoria tritici* blotch (*Mycosphaerella graminicola*) has become an important disease following the introduction of modern germplasm. The high genome plasticity of the pathogen and its specialization features (durum *vs*. bread wheat) complicate the identification of valuable resistance genes with durability and effectiveness across diverse growing areas. The genetic variation of the response to *Septoria* and the chromosomal location of resistance factors were studied using a germplasm collection of 183 durum wheat accessions of diverse origin suitable for association mapping (Maccaferri et al. 2006, Plant Genetics Resources 4: 79-85). The panel was evaluated in 2008 and 2009 in Tunisia (Beja), Mexico (Toluca) and Italy (Argelato and Ferrara). The accessions were then inoculated under controlled conditions with ten *Mycosphaerella* durum wheat isolates collected in a range of Mediterranean countries, as well as with bread wheat isolates and selected strains derived from crosses between durum and bread wheat isolates. The germplasm collection has been genotyped with ca. 300 SSRs of known map position and ca. 900 durum DArT markers. Highly diversified marker-trait association patterns have been obtained based on the field data, isolate and response-trait (% of necrotic leaf area and picnidia production). A preliminary analysis highlighted some chromosome regions consistently involved in *Septoria* resistance, particularly in chrs. 1BL, 2AL and 4AL that accounted for a sizeable portion of phenotypic variation among accessions. The detailed results will be presented and discussed.

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Poster Communication Abstract – 6A.17

DEVELOPMENT OF A TILLING POPULATION IN DURUM WHEAT CV. AUREO

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Wheat, EMS, TILLING, carotenoid pigments

During the last two decades, DNA sequencing has led to availability of gene sequences in key species encouraging the development of alternative strategies to create novel alleles in specific genotypes of crop species. One of these new emerging technologies is the TILLING (Targeting Induced Local Lesions In Genomes) that combines random chemical mutagenesis with high-throughput discovery of the induced mutations in target genes.

In the present study we developed a new wheat TILLING population by treating seeds (cultivar Aureo) with ethyl methanesulfonate (EMS) for mutagenesis. The first experiment was conducted to determine a suitable dose of the EMS needing to achieve at least the 50% of seed survival. Eight sets of 100 seeds of cv. Aureo were treated with eight different doses (0.45, 0.50, 0.55, 0.60, 0.65, 0.70, 0.75, and 0.80) of EMS. It was observed that the survival rate of plants with EMS doses 0.60% was closest to the targeted percent of survival. Thus, the 0.60% treatment was chosen for the development of the TILLING population. To evaluate the mutation densities of the TILLING population represented by 4608 M_2 progenies, we used both a phenotypic and molecular approaches. The phenotypic analysis of M_2 families has been conducted for the determination of germination index, mean time of germination, coleoptiles length and for some morphologic characters (colour of coleoptiles, absence of roots and absence of coleoptiles). Of the total of 4608 M_2 progenies, a first screening was carried out on 200 families (10-20 seeds for each family). An high percentage of the mutant phenotypes was scored because of EMS treatment, indeed comparing to the wild-type genotype, most of the M_2 families presented a delay in germination, reduced power of germination, reduced coleoptiles length, different colour of coleoptiles, variable roots number, and absence of coleoptiles. The molecular approach for revealing the mutation density of TILLING library consisted, instead, in the characterization of two genes involved in carotenoid biosynthesis: *LYC-ε* and *LYC-ß* genes. Both enzymes are involved in the different cyclization of lycopene molecules: *LYC-ε* catalyzes the formation of ε-ε rings in a side of lycopene chain producing α-carotene molecule, while *LYC-ß* generates ß-ß rings to the end of chain producing β-carotene molecule. Non functional genes of *LYC-ε* gene is expected to block or reduce the metabolic flux into the ε branch leading to lutein. While null or less efficient alleles of *LYC-ß* gene is expected to block the next steps of oxygenation, thus leading to accumulation of upstream compounds lycopene towards α-carotene production. The sequences of the two genes has been well characterized and genome-specific primers were designed in order to screen the whole wheat TILLING population.

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Poster Communication Abstract – 6A.18

DEVELOPMENT OF A MARKER ASSISTED SELECTION PROGRAM FOR THE IMPROVEMENT OF DURUM WHEAT (*TRITICUM DURUM* DESF.)

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Plant breeding, durum wheat, marker-assisted selection, gene pyramiding

Italy is the main producer of pasta in the world and the genetic improvement of durum wheat represents a strategic activity for the entire agro-industrial sector.

DNA markers have an enormous potential to improve the efficiency and precision of conventional plant breeding via marker-assisted selection (MAS). This paper describes the work carried out at the Cereal Research Centre (CRA-CER) for the development of a MAS breeding program dedicated to the pyramiding of genes for low lipoxygenase (LOX) activity (*Lpx-B1.1*), high protein content (GPC; *Gpc-B1*), high yellow pigment content (YPC; *Psy-A1*) and disease resistances. The following R genes were considered: leaf, stripe and stem rust (*Lr14c*, *Yr36* and *Sr13* or *Sr26*, respectively), powdery mildew (*Pm36*) and soil borne cereal mosaic virus (SBCMV; *QSbm.ubo-2BS*).

A set of durum wheat varieties and introgression lines carrying the desirable genes were chosen as donor lines, while the recipient line was the Italian durum cultivar PR22D89, characterized by a high gluten quality and good yield.

The crosses were performed separately for each donor line with PR22D89, than the introgressed genes were first fixed in a homozygous after the screening of the F_2 populations. Then, the F_2/F_3 plants homozygous for the same genes and meeting the required phenotypic standards were selected for further crosses in order to combine up to 4 genes of interest segregating in the same populations.

Presently, several hundreds of genotypes are under evaluation and some $F_{3:5}$ lines are carrying genes at the homozygous state combining four different traits of interest: high GPC, low LOX activity, resistance to stripe rust and powdery mildew; as well as high GPC, low LOX activity, resistance to stripe and leaf rust. These lines exhibited a good increase in GPC with a very limited negative impact on grain kernel weight.

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Poster Communication Abstract – 6A.19

FUNCTIONAL MARKERS FOR GRAIN POLYPHENOL OXIDASE ACTIVITY IN A WHEAT COLLECTION

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Polyphenol oxidase activity, wheat collection, functional markers, association analysis

Polyphenol oxidase (PPO) activity in wheat grains causes undesiderable darkening of various end-products. In the last years, the knowledge of genetic control of PPO activity has substantially accelerated development of wheat cultivars with low PPO activity to reduce flour darkening in wheat. One of the most powerful tools to evaluate and validate the association between PPO activity and the flour darkening is the identification of PPO allelic variations and the development of functional markers. In comparison to genomic markers, candidate genes are more powerful because they are directly involved with known biological function or they regulate the developmental processes of the investigated traits, which could be confirmed by evaluating the effects of the allelic variants in association analysis.

The aims of the present study were 1) to analyze the genetic diversity for PPO activity, 2) to develop new genome-specific functional markers for PPO genes and 3) to validate molecular markers for PPO activity in a tetraploid wheat collection.

A collection of 231 tetraploid wheat accessions (*Triticum turgidum* L.), including 128 old and modern cultivars of durum wheat (*T. turgidum* L. var. *durum*) and 103 wild and domesticated tetraploid wheats, was evaluated for PPO activity in kernels. The analysis of variance for PPO activity showed high significant differences ($P \leq 0.001$), with an average of 0.63 (measured at A_{405} nm) ranging between 0.15 and 1.88, and high heritability of the trait ($h^2 > 80\%$).

To obtain B genome-specific sequences, several primers pairs were designated based on the sequence of PPO gene (GenBank Accession Number, GQ303713.1). Two primer pairs, physically mapped on chromosome 2B, amplified a single product of 500-bp and 900-bp, respectively, in the cultivar Chinese Spring.

Some of the reported molecular markers for PPO activity have been analyzed in the tetraploid wheat collection previously described. Interestingly, *PPO18* marker, mapped on chromosome 2A by Sun *et al*., 2005, showed a polymorphic electrophoretic pattern: it amplified a 730 bp fragment in the cultivars with low PPO activity, a 850 bp fragment in the cultivars with high PPO activity, and null allele in several accessions. Association analysis validated that the 730 bp fragments was associated with higher PPO activity than the 850 bp fragments and null allele.

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Poster Communication Abstract – 6A.20

POSITIVE EFFECTS ON YIELD-RELATED TRAITS OF DIFFERENT CHROMOSOMAL SEGMENTS OF THE WILD *THINOPYRUM PONTICUM* INTROGRESSED INTO DURUM WHEAT

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Wheat, chromosome engineering, alien gene transfer, yield, QTL

In order to maximize exploitation of useful genetic variability from the wild wheat relative *Thinopyrum ponticum*, previously developed durum wheat-*Th. ponticum* recombinant lines, carrying the *Lr19+Yp* genes, were subjected to field tests in central Italy (Viterbo) for two years (2009-10). Three recombinant lines, named R5-2-10, R112-4 and R23-1, possessing 23%, 28% and 40%, respectively, of their 7AL arms replaced by *Th. ponticum* homoeologous 7AgL distal segments, were used to assess the expression of several yield-contributing traits and eventually associate to defined 7AgL sub-regions putative yield QTL(s) previously ascribed to the presence of 70% of the alien arm. The recombinant lines used showed differential phenotypes for traits such as seed number/ear, tiller number/plant, above-ground biomass, flag leaf width, and grain yield. Line R112-4, in particular, resulted the most promising genotype in terms of overall yield potential, as its 28% 7AgL chromatin caused significantly increased values for tiller number at heading (+28%) and at harvest (+26%), as well as for biomass (+30%), altogether contributing to a significantly higher yield (+25%), at least in the more favourable conditions of 2010. On the other hand, line R23-1 produced the highest number of seeds/ear (+34% assessed on the first tiller), indicating that in the 7AgL segment present in this line and absent from the two others (28-40% distal 7AL arm) a putative QTL for seed number is located. However, as demonstrated by the remarkably lower yield shown by the same line R23-1, its 7AgL segment does not appear exploitable in durum wheat breeding, presumably due to a negative linkage drag determined by alien Segregation distortion genes. The information obtained represents an essential "first draft" of the identification of yield-related traits associated with 7AgL fragments already incorporated in various recombinant lines of durum and also bread wheat. Combined with the information coming from a dense 7AL/7AgL genetic map concomitantly developed, the possibility that specific markers for the different traits can be detected and hence used in MAS seems a feasible target.

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Poster Communication Abstract – 6A.21

USING WILD SPECIES OF THINOPYRUM GENUS IN BREEDING WHEAT RESISTANT TO FUSARIUM HEAD BLIGHT

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Chromosome engineering, Triticum aestivum, Triticum durum, FHB resistance, scab

In recent years, climatic changes have favoured the spreading of previously uncommon fungal diseases, including Fusarium Head Blight (FHB), in several wheat growing areas worldwide. Because of the relevant economic damages to wheat production and quality caused by FHB, and the scarcity of resistant sources among cultivated *Triticum* species, we have looked outside the primary gene pool and targeted wild wheat relatives of the *Thinopyrum* genus as promising sources of effective resistance. Two such species are the decaploid *Thinopyrum ponticum* (carrier of an *el*-type genome) and the diploid *Thinopyrum elongatum* (carrier of an E-type genome). In both species, one or more genes/QTLs for FHB resistance are located on the long arm of a homoeologous chromosome to those of wheat group 7 (named 7el_2L and 7EL, respectively). Resorting to chromosome engineering, i.e. aiming at the recombination-based transfer into wheat of small, 7el_2L or 7EL chromosomal segments carrying the desired gene(s), we were aware of the different cytogenetic affinity relating the donor and the recipient chromosomes. In fact, bread wheat substitution lines for the 7el_2L arm or the complete 7E chromosome for the wheat 7D counterparts were employed as donor lines, while durum and bread wheat recombinant lines, already carrying *Th. ponticum* 7el_1L portions, have been chosen as recipient lines. These contain the 7el_1L-derived *Lr19+Yp+Sr25* genes but lack any effective FHB resistance gene. Almost complete homology has been verified to exist between 7el_1L and 7el_2L (presumably originating from different *Th. ponticum* accessions), whereas that between the former and 7EL of *Th. elongatum* is only partial (around 20% pairing as from our records). Thus, homologous recombination is expected to allow pyramiding of all the desired 7el_1L + 7el_2L traits with relative ease, while the use of wheat homoeologous pairing mutants (*ph1* and *ph2* mutations) is being included in the transfer schemes to enhance 7el_1L-7EL recombination frequency. The multi-targeted and multi-genomic transfers are being aided by development of suitable polymorphic markers along the 7L arms and application of GISH (Genomic In Situ Hybridization) in somatic and meiotic cells. As a result, the first recombinants with the desired gene combinations are being isolated.

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Poster Communication Abstract – 6A.22

HIGH-THROUGHPUT GENOTYPING AND COMPARATIVE GENOMICS APPROACHES FOR MAP BASED CLONING OF *UNICULME4*, A MENDELIAN LOCUS CONTROLLING BARLEY SHOOT ARCHITECTURE

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Hordeum vulgare, tillering, uniculme4, positional cloning, TriticeaeGenome

Manipulation of plant architectural traits such as the number of tillers can effectively increase grain yield in cereals. *Uniculme (cul)* recessive loci including *cul4* are required for tillering in barley. As participants in the FP7 TriticeaeGenome project (http://www.triticeaegenome.eu/), an objective of our group is the fine mapping and positional cloning of the *cul4* gene. Initial efforts were based on genotyping of six F2 populations deriving from crosses of *cul4.3, cul4.5,* and *cul4.15* mutants with wild-type cultivars using an Illumina GoldenGate assay: 96 EST-derived SNP markers covering a 50 cM interval on 3HL were selected starting from an initial set of 8 SNPs identified by comparison of a *cul4* introgression line and its recurrent parent. As a result, the *cul4.5* x Morex segregating population was selected as the most informative cross and propagated into >4900 F3 plants for positional cloning. The large F3 population was genotyped with tightly linked SNP markers using the KASPar genotyping system resulting in the identification of 179 recombinants around *cul4*. Phenotyping and genotyping of these plants with 8 new markers developed from collinear Brachypodium and rice genomic regions allowed the identification of a candidate gene cosegregating with *cul4* as well as mapping of the two flanking genes in Brachypodium 0.11 cM and 0.12 cM from *cul4,* defining a 0.23 cM interval around the locus. Comparative genomic analysis with these three gene-based markers identified 27 kb and 30 kb

collinear regions in rice and Brachypodium, respectively. Physical mapping of the *cul4* gene region is underway taking advantage of the available barley physical map.

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Poster Communication Abstract – 6A.23

r1 GENE IS TIGHTLY ASSOCIATED TO A QTL INVOLVED IN MAIZE YIELD

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Zea mays, r1 gene, anthocyanins, QTL analysis

Anthocyanins are a class of water-soluble molecules produced only in plants and conferring a red-blue colour depending by the vacuole pH where they are stored in glycosylated form. These substances play important roles in several physiological processes such as UV protection, male fertility, antimicrobial activity and in general they are involved in protection against oxidative damage. In maize, anthocyanins are synthesized by a pathway made up by about 20 genes activated by transcription factors belonging to two gene families: *c1/pl1/p1* (MYB genes) and *r1/b1* (bHLH genes). Anthocyanin accumulation in a specific tissue requires the presence of a member of both families: from the MYB family, *Pl* is required for pigment accumulation in the plant and *C1* acts in the aleurone while *r1/b1* acts in the seed and plant tissues depending on the nature of the alleles present.

In this work we produced and studied for three years two synthetic populations of maize differing in their constitution only for the selected alleles present at the *red color 1* (*r1*) locus (*R-sc vs r-r*). The *R-sc* allele confers pigmentation only in the aleurone seed layer, while the *r-r* allele confers pigmentation in several tissues such as root, silk and anther but the seed is colourless. The colourless population (*r-r/r-r*) was characterized by improved agronomic features such as ear weight and plant height compared with the *R-sc/R-sc* coloured population. This finding was confirmed studying single F_4 *R/r* families where the presence of the *r-r* allele conferred positive features, acting as a dominant trait.

Quantitative trait locus (QTLs) analysis performed using molecular markers on the long arm of chromosome 10 (bin 10.06) where the *r1* gene maps, identified a QTL map position for plant height tightly associated to the *r1* gene. Thus the *r1* gene may represent a major QTL or it could be closely linked to another gene involved in the agronomic performance of the two populations studied.

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Poster Communication Abstract – 6A.24

AUXIN EFFLUX TRANSPORTERS IN MAIZE: PHYLOGENETIC ANALYSIS AND GENE EXPRESSION STUDIES

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Auxin, development, Polar Auxin Transport, PIN auxin efflux carriers, Zea mays

Auxin regulates many aspects of plant development, such as embryogenesis, leaf, flower and lateral root initiation, vascular tissues differentiation and tropisms. Auxin molecules can move over long distances through the vascular system via the phloem, from source tissues to the roots. However, it is well known that a polar cell-to cell transport is the main mechanism involved in the wide spectrum of auxin-related developmental processes. Multiple transport proteins are required to create and maintain directional auxin flows within organs and tissues: the resulting auxin concentration gradients are essential for the establishment and maintenance of polar growth and morphological patterning. A better understanding of auxin transporter role in mediating polar auxin transport (PAT) in maize is of outstanding importance for basic plant biology research and for crop improvement as well: shoot and root architectures, two of the main issues in determining crop yield, are indeed strictly regulated by PAT.

To date in Arabidopsis, three families of auxin transport proteins have been identified: AUXIN RESISTANT 1/LIKE AUX1 (AUX1/LAX) uptake symporters, P-GLYCOPROTEIN (MDR/PGP/ABCB) transporters and PIN-FORMED (PIN) efflux carriers.

In our group we identified the twelve members of the maize *PIN* gene family and two *PIN-like* genes. In this poster we report the characterization of the maize *PIN* and *PIN-like* genes the analysis of their expression patterns during pre- and post-embryonic development and their localization in different maize tissues during differentiation and development. Our results confirm the widening of monocots *PIN* family compared to dicots one (twelve maize *PINs* versus the eight Arabidopsis members) showing also in maize, as previously reported in rice and sorghum, the presence of three monocot-specific proteins, namely ZmPIN9, ZmPIN10a and ZmPIN10b. This indicates the fundamental role of PIN efflux-driven auxin accumulation for proper monocots development. Some *ZmPINs* show overlapping expression patterns, suggesting a certain degree of functional redundancy, whereas other family members, for example *ZmPIN9*, present peculiar expression domains. Given that, the preliminary data on possible molecular mechanisms regulating *ZmPIN9* expression will be illustrated.

Furthermore, to better elucidate the role of PAT in maize root architecture we are currently investigating the role of BR2, a maize MDR/PGP/ABCB efflux transporter expressed in internodes and roots, using a *br2* mutant. The mutant plants show a slow response to changes in the gravity vector, resulting in the formation of agravitropic primary roots.

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Poster Communication Abstract – 6A.25

DEEP GENOME -WIDE CHARACTERIZATION OF RECOMBINANT NEAR-ISOGENIC LINES FOR HETEROTIC QTL IN MAIZE

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Near isogenic lines, QTL, heterosis, SNP

In a previous study on a maize (*Zea mays* L.) population of Recombinant Inbred Lines (RILs) derived from B73 x H99, we detected several Quantitative Trait Loci (QTL) for agronomic traits and with a high dominance ratio (heterotic QTL). Then, to gain a better insight on the effects of such QTL, we developed two pairs of near-isogenic lines (NILs) for QTL at bin 3.05, two pairs of NILs for QTL at bin 4.10 and one pair for QTL at bin 10.03. For each pair, the two NILs should contrast for the parental genotypes at the corresponding QTL region (NIL-BB and NIL-HH if homozygous either for the QTL allele provided by B73 or by H99, respectively), while should share in homozygosity the rest of the genome for the alleles provided by both parents. This work was conducted on these five pairs of NILs to characterize them at about 50,000 genome-wide distributed SNPs by the Illumina MaizeSNP50 Genotyping Bead Chip platform.

After general data quality check, 14,937 (34.9%) of the 42,776 good quality SNPs were found polymorphic for the parental alleles. The observed low residual heterozygosity in NILs (average 0.93%) confirmed the success of the inbreeding program employed for QTL introgression. The proportion of SNPs in homozygous state for the B73 allele in NILs ranged from 28.5% (NIL4.10_R55-B) to 51.3% (NIL3.05_R8-B), with an average of 39.4%. Plotting the pattern of inherited genotypes and identity-by-state (IBS) values of single SNPs against their chromosome position allowed to precisely map all recombination blocks in NILs as well as to identify all genomic regions harbouring different genotypes between NILs within the same pair. This analysis confirmed that the selected QTL regions were successfully introgressed as expected in all NILs. Moreover, the dense and genome-wide SNP coverage allowed us to identify undesired chromosomal regions unexpectedly segregating between NILs belonging to the same pair, which had gone undetected by all previous analyses. A NIL-F2 segregating population derived from one of the NIL pairs (4.10_R55) for fine mapping purposes is currently being scored by selected SSR markers in order to include and evaluate the effect of allele substitutions at all segregating regions on the phenotypic values previously associated with the introgressed QTL only.

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Poster Communication Abstract – 6A.26

SEED DEVELOPMENT AND IAA BIOSYNTHETIC GENES ARE REGULATED DIFFERENTIALLY IN THE DEFECTIVE ENDOSPERM-18 SEED MUTANT OF MAIZE

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Endoreduplication, endosperm, auxin biosynthesis, Zea mays, q-PCR

The *defective endosperm*-18 *(de*18*)* mutant accumulates substantially less dry matter in the endosperm than its normal counterpart. The auxin IAA levels in *de*18 endosperm are 15 times lower respect to the wildtype. The addition of synthetic auxins to the developing *de*18 grains rescues the wild type phenotype. Previous studied showed that auxin is involved in enhancing post-mitotic nuclear DNA synthesis (endoreduplication), that is positively correlated with cell enlargement and cell volume. We have investigated whether the reduced endosperm of *de*18 is due to impaired cell division and endoreduplication process, as a consequence of the low auxin levels. Nuclear endoreduplication level, number and size of cells have been measured in wild-type B37 and *de*18 kernels at 8, 12, 14 and 16 DAP with the optical miscroscope and computer image analysis, using the 3D model developed for maize endosperm. Observations of cells distribution with different ploidy levels in both genotypes, showed that at 8 DAP most of cells in the endosperm were 3C and 6C cells and they were restricted mainly to the outermost layers. Endoreduplication began in the nuclei of the central starchy endosperm cells (12C) and proceeded basally and outward until 16 DAP, where 96C and 192C nuclei were localized in the central part of endosperm. The most significant differences between *de*18 and B37 were detected at 8 DAP, where the mutant showed a deficiency in the ploidy level, number and volume of cells. The expression level of auxin regulated genes appears to be reduced in *de*18 during endosperm development, as revealed by q-PCR analyses. A comparative analysis of expression of five genes involved in IAA biosynthesis showed significant alterations of expression in the mutant than the wild type at different stages of endosperm development.

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Poster Communication Abstract – 6A.27

EXPLORING DROUGHT RESISTANCE IN TEMPERATE RICE FOR A SUSTAINABLE RICE PRODUCTION IN ITALY

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Rice, drought, genomics, stress response, genome-wide association

During the last two centuries rice in Italy developed essentially as a water demanding crop, completing the growth cycle under submersion. Reduced water availability due to climate changes, especially if occurring during critical phases of the plant growing cycle, dramatically affects crop yield and quality.

This study aims at understanding the mechanisms leading to drought resistance in temperate japonica rice. Drought resistance is a complex phenomenon comprising a number of morpho-physiological processes at different plant developmental stages, resulting in the capability to withstand scarce water input and/or lack of water for longer periods while maintaining yield stability. The root-system architecture plays a crucial role in conferring drought tolerance. A deep root system able to absorb water at depth is the most relevant trait contributing to drought avoidance in upland conditions.

In this work, the existing biodiversity in a collection of 100 temperate japonica rice varieties will be characterised in terms of drought tolerance through the most advanced genomic tools coupled to phenotypic evaluations in growth-controlled and in field conditions. The collection includes traditional and modern accessions representing the genetic diversity of the Italian rice germplasm and a set of foreign varieties from temperate areas adapted to Italian climatic conditions. A phenotypic screening for root morphological features will be performed in controlled greenhouse conditions, while growth and yield performances will be field-evaluated under standard (submergence) and water-limited (aerobic soil) conditions.

In parallel, the same set of varieties will be genotyped using a high density 600K SNP panel. Genome-wide association analysis will then be carried out to identify new alleles and molecular markers to pin point the genetic determinants controlling drought resistance in Italian rice.

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Poster Communication Abstract – 6A.28

A COMPARISON OF METHODS FOR ASSOCIATION MAPPING IN RICE

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Oryza sativa, Mixed Linear Model, Random Forest, association mapping, SNP

The main objective of this study was to compare alternative methods for association mapping with those implemented in the software package TASSEL, which is commonly used in plant association studies. An empirical rice data set was used which comprised phenotypic (14 morpho-physiological traits) and genotypic (383 SNPs selected in 150 target genes) data of 152 accessions of *O. sativa*.

Three methods available in R packages and a JAVA-based software (RanFog) were tested and an ad-hoc pipeline was designed to run the association analyses using R/Java scripts.

All those methods adjust for complex population structure among genotypes, which induces inflated false positive rates. The first two methods tested (EGSCORE and GRAMMAR) were taken from the R package GenABEL and are based upon a mixed model approach. The former adjusts for possible stratification by principal components, while the latter copes with the problem of population structure by using a marker-based kinship matrix. In both cases phylogenetic information obtained by STRUCTURE software was added to the mixed model as a fixed effect. The third method, EMMA, was taken from the R package EMMA (Efficient Mixed-Model Association). This method takes into account the population structure (as fixed effect) and is similar to Grammar, correcting for population structure by means of a marker-based kinship matrix. The fourth method used was a Java implementation of the Random Forest algorithm. One of the main advantages of this method is the speed of analysis, which would become more relevant in case of association studies using high-density SNP panels (GWAS).

Using the results obtained from TASSEL as the reference set, we found that EMMA attained the best performances giving the higher number of SNPs in common with TASSEL significantly associated with the analyzed traits.

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Poster Communication Abstract – 6A.29

IDENTIFICATION OF TOMATO DIFFERENTIALLY EXPRESSED GENES INVOLVED IN RESPONSE TO WATER DEFICIT

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Solanum lycopersicum L., water deficit, gene expression analysis

Environmental stress adversely affects plant performance, resulting in significant reduction of crop yield and quality worldwide. Increasing aridity of semi-arid regions together with limited water resources has led to an exigent necessity for improving crop drought resistance.

Elucidating the molecular mechanisms of drought tolerance is critical for increasing crop production and quality. Plants do not passively accept environmental stresses, but respond actively through perception of stress signals. Responses to water deficit may occur within a few seconds or within minutes and hours when a plant withstands the imposed stress, and may arise from either tolerance or mechanisms that permits avoidance of the situation. In trying to understand responses to stresses, many genes induced by periods of water deficit have been identified and characterized. Interest has centred on differentially expressed genes, because it has been postulated that induction of genes will permit adaptation to stresses.

We present results of a study on tomato (*Solanum lycopersicum* L.) differentially expressed genes induced by water deficit. Two genotypes, selected based on drought tolerance and susceptibility, were grown in "semi-controlled" conditions (i.e. outdoor and protected from rainfall by transparent plastering covering), with three water stress treatments, and in greenhouse with two water stress treatments. We investigated the expression of 15 genes candidate to be involved in response to water deficit One gene, ERD15, showed a differential expression profile between[0] the two genotypes grown under the "semi-controlled" conditions, while 5 genes, including the same ERD15, were differentially expressed in greenhouse.

We are currently carrying out a proteomic approach to further analyze genotypic drought responsiveness.

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Poster Communication Abstract – 6A.30

CONDITIONAL EXPRESSION OF THE TOMATO PROLINE TRANSPORTER LeProT1 CONFERS TOLERANCE TO HEAT AND OTHER ABIOTIC STRESSES

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*HSP 18.2 promoter, heat stress, drought, salinity, tomato proline transporter (*LeProT1*)*

High temperature stress is becoming a major problem because of predicted increase of 2°C of earth surface temperature by 2050. Increased temperature is a problematic factor for both vegetative and reproductive development of crop plants. Exposure to high temperature causes reduced yields in tomato (*Solanum lycopersicum* L.), that are mainly due to adverse effects on male gametophyte development. Proline protects membranes and proteins against temperature extremes and functions as a hydroxyl radical scavenger. It was demonstrated that the proline content of anthers plays important roles in acquiring heat tolerance in tomato, but proline transport to anthers is seriously impaired by heat stress. This leads to high proline accumulation in leaves (source) instead of developing pollen grains (sink). In this research, we engineered tomato plants to express the endogenous anther-specific proline transporter *LeProT1* under the control of the heat-inducible *HSP 18.2* promoter from Arabidopsis, in order to increase proline supply to developing anthers under heat stress. Primary transformants and control plants (cv MicroTom) were subjected to a heat stress of 38°C for 2 h at about 3 d before anthesis of the first flowers. Transformed plants performed better than control plants for pollen stainability (94.0% vs 81.1%) and germinability (91.6% vs 74.6%). This was reflected into a significant difference in yield between untransformed control plants and plants engineered with the *LeProT1* gene. These phenotypes of increased heat tolerance were positively correlated with the proline content in the developing anthers of transformed plants. Because proline is believed to act as an osmoprotectant also in plants subjected to drought and/or salt stresses, we checked if heat shock-driven activation of the *LeProT1* proline transporter could positively affect the proline metabolism and the development of seedling subjected to water deficiency or salinity. Experiments on seedlings showed that transformed genotypes performed better also under drought and salinity stress. As these latter stresses are often correlated with heat, the engineering of the *LeProT1* expression in tomato represents a useful strategy to increase abiotic stress tolerance and increase crop yields under this present arena of global warming.

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Poster Communication Abstract – 6A.31

ADVANCES IN THE CHARACTERIZATION OF TOMATO MUTANTS PUTATIVELY AFFECTED IN CLASS B MADS-BOX TRANSCRIPTION FACTORS

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Deficiens, Globosa, male sterility, Solanum lycopersicum, tomato

The interested towards male sterile mutants in tomato dates back long time ago due to the perspective of using male sterility (MS) in hybrid seed production. In order to exploit genic MS, the selection of conditional sources, where sterile anthers are be restored to fertility by permissive growth conditions or by the application of appropriate growth regulators, has been regarded as useful strategy. Therefore, mutants with conditional expression, such as *stamenless* (*sl*), *stamenless-2* (*sl-2*), *7B-1* and *variable male sterile* (*vms*), have been deeply studied in the past. To identify the genes underlying these mutations would be very important in order to pursue the discovery of new alleles by the screening of large mutagenized populations. In this research we describe our recent advances towards this goal. Literature data and examination of the phenotype indicated as candidates for these mutations members of the class B MADS-box transcription factors family, that specify the identity of the second (petals) and third (stamens) floral whorl. In *Arabidopsis thaliana* and *Antirrhinum majus*, this class of genes is composed of only two members that, referring to the *A. majus* nomenclature, are known as *DEFICIENS* (*DEF*) and *GLOBOSA* (*GLO*). In tomato, as in most solanaceae and in other families, these transcription factors both underwent a duplication event followed by subfunctionalization, leading to two *DEF*-like members (*SlDEF* and *SlTM6*) and to two *GLO*-like members (*SlGLO1* and *SlGLO2*). Through genetic analysis, we confirmed that the *sl* and *sl-2* mutations are allelic and demonstrated that *7B-1* is also traceable to the same locus. Surprisingly, analysis of two independent mapping populations, one segregating *sl-2* and one segregating *7B-1*, excluded the involvement of these mutants in the *SlDEF* locus on the long arm of chromosome 4, as was earlier reported in the literature. The flower phenotype of the two mutants, similar in that they show nearly normal petals and heavy carpellization of the anther cone, indicates *SlTM6*, the paralog of *SlDEF*, as a better candidate for the *Sl*/*7B-1* locus. Experiments with new mapping populations to confirm this hypothesis are under way. For the *vms* mutant, mapping experiments indicated that it co-localizes with the *SlGLO1* locus on the long arm of chromosome 8. Sequencing of *SlGLO1* in the mutant and in its nearly isogenic wild-type revealed that the mutant carries a transition leading to an E123K residue substitution in the K-domain, a region under strong purifying selection that is involved in protein–protein interaction.

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Poster Communication Abstract – 6A.32

PRELIMINARY ANALYSIS OF *AN1* MYB GENE IN WILD AND CULTIVATED POTATO SPECIES

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Anthocyanins, transcriptional factors, MYB, Solanum

In the plant kingdom there are three major compounds that confer colours to the plants: anthocyanins, carotenoids and chlorophylls. Among all, anthocyanins are of outstanding interest for their well-documented beneficial effects on plant physiological processes and human health. Indeed, anthocyanins are generally accepted to be enhancers of plant reproductive success as well as plant defence mechanisms. Studies on the anthocyanin synthesis pathway discovered all the structural enzymes involved and the transcription factors that regulate their activities. Among them, a member of MYB TFs gene family, named *an1* in potato, seems to be the key player in the anthocyanin accumulation. The aim of this study is to characterize the complete gene structure (CDS, exon/intron, promoter) of *an1* in potato and study its expression in resistant wild potato species. We analysed both white and purple-skin genotypes of *Solanum tuberosum* and two wild potato species, *S. commersonii* and *S. bulbocastanum,* carrying noteworthy resistance traits. By using specific primers we were able to amplify fragments from genomic DNA of all genotypes analyzed. Differences in PCR patterns, likely due to different allelic isoforms present in wild potato species, were found. Preliminary results on shared bands confirmed the identity of both *S. tuberosum* and *S. commersonii* amplicons to *an1* gene. Sequence analysis of alternative isoforms is ongoing. Our future work will focus on the analysis of *an1* gene expression in wild potato species challenged with different biotic and abiotic stresses to understand the role of anthocyanin in plant defence mechanisms.

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Poster Communication Abstract – 6A.33

DEVELOPMENT OF AN SNP-BASED GENETIC LINKAGE MAP AND QTL ANALYSIS IN EGGPLANT

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Eggplant, Restriction-site Associated DNA (RAD), genetic linkage map, QTL, horticultural traits

The eggplant (*Solanum melongena*) genome is relatively unexplored, especially compared to those of the other major *Solanaceae* crops tomato and potato. Genetic maps based on both inter-specific and intra-specific crosses have been developed in the last years. The most recent inter-specific map is constituted of 347 COS and RFLP markers spanning 1,535 cM, while the 2 most recent intra-specific maps comprise 238 markers and 236 markers, spanning 718.7 and 951.4 cM respectively. The level of marker saturation is however still low for fine mapping and genomic synteny studies. We recently combined the developed Restriction-site Associated DNA (RAD) approach with Illumina DNA sequencing to effect the rapid and mass discovery of both SNP and SSR markers for eggplant (1).

A subset of 384 SNPs was used to genotype an F_2 intraspecific mapping population and integrated into a previously developed genetic linkage map (2) encompassing a total of 426 markers, including 343 SNPs, 43 SSRs, 28 COSs, 11 RFLPs and 1 CAPS. The framework map included 418 markers spanning 1,388 cM with an average map distance of 3.32 cM. Thanks to RFLP and COS markers, the chromosome assignment of all the linkage groups (LG) identified was performed. The length of eggplant chromosomes ranged from 152 cM (E6) to 78.6 cM (E8).

The newly developed map was then used for performing QTL analyses on several agronomical traits related to fruit quality, plant architecture and plant development, phenotyped in two locations. Preliminary results are available for traits as fruit weight, fruit shape and anthocyanins content in leaves, revealing the identification of at least one major QTL for each of these traits. Further ongoing analyses are allowing a comparison of the QTLs identified in the two environments, thus making possible a reliable identification of genomic regions involved in the control of key horticultural traits.

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Poster Communication Abstract – 6A.34

FIRST RESULTS ON THE OVEREXPRESSION OF *Cs*GSTU ISOENZYMES IN TRANSGENIC TOBACCO PLANTS

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Citrus sinensis, glutathione transferase, detoxification, transgenic plant

The glutathione S-transferases (GSTs, EC 2.5.1.18) are members of a multifunctional superfamily of enzymes catalyzing the conjugation of glutathione (GSH) to the electrophilic groups of hydrophobic and usually cytotoxic molecules of either endogenous or exogenous origin. GSTs are widely distributed in nature and are found in several organisms from humans to bacteria. In plants the GSH addition reaction is coupled to the vacuolar compartmentation of the GS-conjugates because of the lack of an effective excretion pathway which is, instead, active in animals. Based on protein sequence similarity, active site residue and gene organization plant GSTs are grouped in four main classes (phi, tau, zeta, theta). The majority of the plant GSTs belongs to the tau (GSTU) and phi (GSTF) classes which are plant specific. Among the plant GST classes, tau is the most numerous and members of this class overlap in their function of enhancing crop stress tolerance. Transgenic plants overexpressing GST subunits active in herbicide detoxification confirmed the GST's role in crop's herbicide selectivity, and, the down-regulation of GST subunit active in the detoxification process can result in reduced tolerance to herbicide of the transformed crop. In a previous study we isolated from sweet orange leaves [(*Citrus sinensis*) L. Osbeck)] two GST genes, namely GSTU1 and GSTU2. The encoded proteins differ only for three amino acids all of them included in the C-terminal domain of the enzymes (R89P, E117K, I172V). In order to understand the significance of the single mismatched residues between U1 and U2 (R89P, E117K and I172V, respectively) site-directed mutagenesis experiments were undertaken to generate several mutate enzymes. Among the mutate enzymes, GST-RKV, obtained by the substitution R89P upon the isoform GSTU2, showed extremely high catalytic efficiency towards GSH and pronounced ability to conjugate GSH to the alkyl halide 4-nitrophenethyl bromide, a molecule of toxicological interest in view of its occurrence as environmental pollutant. Due to these features GST-RKV exhibits great potential for the development of germplasm with novel favourable traits. As a consequence, we report here the first results on generation via *Agrobacterium* transformation of transgenic tobacco plants overexpressing both *Cs*GST isoenzymes and mutants, and *in planta* study of the their role in detoxifying herbicides.

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Poster Communication Abstract – 6A.35

EXPRESSION OF A METALLOTHIONEIN A1 GENE OF *PISUM SATIVUM* IN WHITE POPLAR ENHANCES TOLERANCE AND ACCUMULATION OF ZINC AND COPPER

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Antioxidant enzyme, heavy metal, metallothionein, transgenic poplar, white poplar

Metallothioneins (MT) play an important role in heavy metal detoxification and homeostasis of intracellular metal ions in plant. In this study, two transgenic lines expressing the $PsMT_{A1}$ gene from *Pisum sativum* for a metallothionein-like protein, a regenerated non transformed line NT and the clone AL22, selected as heavy metal tolerant, were characterized for the ability to accumulate zinc and copper and to activate antioxidative enzyme defenses, superoxide dismutase, catalase, ascorbate peroxidase, in presence of the heavy metals (HM). Transgenic line during HM stress showed a higher ability than NT and AL22 to translocate both metals from root to shoot accumulating high amounts of zinc and copper. The antioxidant enzyme defence was differently activated in response to metals in the transgenic lines without a significant increase of reactive oxygen species (ROS). These data suggest that $PsMT_{A1}$ expression decreases ROS accumulation leading to increased zinc and copper sequestration in root and leaf and enhanced metal tolerance.

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Poster Communication Abstract – 6A.36

A DELETION IN THE *ent-KAURENOIC ACID OXIDASE1* (*HaKAO1*) GENE AFFECT THE *dwarf2* (*dw2*) MUTANT OF SUNFLOWER

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Aberrant mRNA-splicing, ent-kaurenoic acid oxidase, gibberellin biosynthesis, mutant

Dwarf mutants in plants are crucial to elucidate regulatory mechanisms for plant growth and development. This character is also favored in breeding [Hedden, (2003) Trends Genet. 19: 5-9]. Identification of the genes responsible for these traits shown that they control gibberellins (GAs) metabolism and/or perception. A dwarf mutant, *dwarf2* (*dw2*) of sunflower (*Helianthus annuus*), showed an extreme reduced size of stem, leaves, petioles and flower organs and a retarded flower development. Pollen and ovules were produced but most disk flower failed to open. The *dw2* phenotype was mainly because of reduced cell size. The mutant responded to the application of bioactive GAs. In *dw2* seedlings, the levels of *ent*-7α-hydroxykaurenoic acid, GA_{19}, GA_{20} and GA_1 were severely decreased relative to those in its wild type (WT). *ent*-Kaurenoic acid was actively converted to *ent*-7α-hydroxykaurenoic acid in WT plants but quite poorly in *dw2* plants. All together these data suggested that the *dw2* mutation severely reduced the flux through the biosynthetic pathway leading to active GAs by hampering the conversion of *ent*-kaurenoic acid to GA_{12}. Two *ent*-kaurenoic acid oxidase (*KAO*) genes were identified. *HaKAO1* was expressed everywhere in sunflower organs, while *HaKAO2* was mainly expressed in roots. The *HaKAO1* of *dw2* displayed an ample deletion (403 nucleotides) encompassing partial sequences of the last intron, the entire last exon and a partial sequence of 3'-UTR. Consequently, the AG required for the positioning of splicing was lost from the last intron. This mutation leads to aberrant processing of the resultant pre-RNA. [Fambrini et al., (2011) Plant Mol. Biol. 75:431-450]. In *dw2* calli, *Agrobacterium*-mediated transfer of WT *HaKAO1* cDNA restored the WT endogenous levels of GAs. In segregating BC_1 progenies, the deletion co-segregated with the dwarf phenotype. The deletion was generated near to a breakpoint of a more complex chromosome rearrangement.

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Poster Communication Abstract – 6A.37

GENETIC DIVERSITY FOR THE RESPONSE TO EXTERNAL STIMULI AFFECTING PHYSIOLOGICAL MECHANISMS IN *HELIANTHUS TUBEROSUS* CLONES

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Jerusalem artichoke, biomass, biotic stresses, abiotic stresses, molecular markers

Helianthus tuberosus L. (2n=6x=102) (*Ht*), is known for possessing important agronomic and technological traits such as the high nitrogen and water use efficiency, competitive ability against weeds, good resistance to fungal pathogen infection, and fructan-rich biomass. Those features make *Ht* a relevant "non food" bioenergy crop for low-input cropping systems.

Few information are available on the response of *Ht* to external stimuli affecting physiological mechanisms relevant for the biomass yield under different environmental conditions.

Ht clones derived from both the improved primary gene pool (GP-1*i*) and the wild primary gene pool (GP-1*w*) were compared to evaluate differential response to several external stimuli, such as: *i*) fungal infection determining powdery mildew (caused by *Erysiphe cichoracearum* or *Ec*), alternaria leaf spot (caused by *Alternaria alternata* or *Aa*), tuber rot (caused by *Sclerotinia sclerotiorum* or *Ss*), *ii*) drought stress in the field , *iii*) GA_3 treatment for tuber breaking dormancy, *iv*) mechanically-induced injury of the tuber apical bud, and *v*) exposure to intensive thermal units administration in the greenhouse. The GP-1*i* clones were obtained from the "Violet de Rennes" (VR) and the Hungarian (CU-3B) accessions, and from the half-sib progenies produced by the 'K8' and 'D19' parental accessions (the monostem clones K8-HS142 and D19-HS2). The GP-1*w* clone CSR was sampled from an ecotype growing along a road-side near Ronciglione in the Lazio Italian region.

Tuber and stem biomass yields were evaluated from the treated and control plant materials of each clone. Statistical tests of the differences among clones for biomass yield was performed for each experiment.

The results demonstrate very clearly that: (*i*) VR was resistant and the other clones were susceptible to *Ec*, *Aa* and *Ss* infections; (*ii*) K8-HS142 displayed a relevant resistance to drought (expressed as biomass yield at the time when foliar senescence extended to the nodes in the lower 50% of the main stem) compared to other clones; under irrigation dry matter was 3.1 kg m^{-2} for K8-HS142 and 2.0 kg m^{-2} for CU-3B; (*iii*) dormant tubers from D19-HS2 and K8-HS142 sprouted faster than tubers from CSR, VR and CU-3B after immersion for 1 hour in a 1 mM GA_3 solution; *iv*) only basal buds on the tubers of the D19-HS2 and K8-HS142 clones sprouted as result of the loss of the apical dominance due to the injury on the tuber terminal bud; *v*) D19-HS2 and K8-HS142 clones displayed 75 days flowering earliness compared to the other clones, when exposed to either regular daily thermal units in the field or to the more intense (+3,5 °C over the external temperature) accumulation of thermal units in the greenhouse; flowering of the earliest clones was anticipated of 60 days when grown in the greenhouse.

Monomorphism observed for the amplicons from the PCR using primers designed from the nucleotide sequence of either the cloned *Ss* resistance gene from *Helianthus annuus* or one of the two key enzymes involved in the fructan biosynthesis (1-FFT), suggested that in some instances, divergent response of the clones to external stimuli may depend on differential modulation of gene expression rather then to polymorphism for the ORF of the involved genes.

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Poster Communication Abstract – 6A.38

IN VITRO MICROTUBERIZATION FOR SIMULATING THE DEVELOPMENTAL PHYSIOLOGY OF UNDERGROUND STORAGE ORGAN IN *HELIANTHUS TUBEROSUS*

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Nodal explants, cold treatment, sprouting, microtubers, Jerusalem artichoke

Production of *Helianthus tuberosus* L. (*Ht*) microtubers by tissue culture, might become a model for simulating the developmental physiology of underground storage organ formation, and a tool for early selection strategy for tuber traits. In particular, carbohydrate levels at microtuber physiological maturity may give insight on the possible relation to the carbohydrate content of mature tubers in the field. Other microtuber traits (time to induction, color, shape) might reflect the genetic diversity among clonal explants for the physiological ability to microtuber differentiation. Microtubers may also be considered as the material of choice for the greenhouse production of disease-free plantlets to propagate *Ht* clones. There are no reports on the proper procedure for *in vitro* microtuber induction in genetically different *Ht* clones, although a general protocol has been proposed.

The method described here allows microtuber induction on single nodal explants dissected from *in-vitro* grown plantlets from tubers of three *Ht* clones: Violet de Rennes (VR), Ungheria-3B (CU-3B), and K8-HS142. Field-grown tubers were surface sterilized with 0.5% sodium hypochlorite, rinsed with sterile water, sliced in disks containing at least one bud, and treated with a BAP/GA_3 solution at 0, 1, 10 and 50 ppm, and placed in the dark for two days. Emerged shoots were detached by cutting with a sterile blade and transferred to sterile jars containing autoclaved proliferation medium where they were subcultured until a constant rate of proliferation was reached. The proliferation medium consisted of Murashige and Skoog (MS) basal salts and vitamins, BAP 0.5 mg L^{-1}, NAA 0.05 mg L^{-1}, sucrose 3%, agar 0.6%, and activated charcoal at 1%. For the microtuberization trials, shoots were divided into segments containing one node each. Four microtuberization agarized media were tested; they consisted of MS basal salts and vitamins, at pH 8, each one containing one of the four combinations of BAP (0 and 0.5 mg L^{-1}) and sucrose (6% and 8%). The node explants of the three clones were subcultured in the four media and were maintained in the dark at 18°C until the microtubers appeared. Microtuberization took place with high success in the VR and CU-3B clones, and it was less conspicuous in the K8-HS142 clone. The presence of BAP in the media improved microtuber formation but it was not an effective factor for microtuber induction. Microtuberization occurred after 1.5 months on VR nodal explants, and after 2 and 2.5 months on the CU-3B and K8-HS142 explants, respectively. Microtubers formed on VR explants were violet in color and pear-shaped, beige and round for CU-3B, and beige and elongated for K8-HS142. Microtubers from K8-HS142 were susceptible to dehydration. The time elapsed for microtuber formation and their color and shape, paralleled the expression of similar traits for the tubers formed by the field-grown plants. Microtubers were stored for two months at +4°C, without loosing dormancy. They sprouted only with a treatment with 0.1% GA_3 for 10 min., without a cold

pretreatment. Microtubers from the control group (no cold pretreatment, no immersion in GA_3), expressed a significant lower percentage of sprouting. The plantlets, obtained from all the sprouted microtubers, displayed a normal phenotype, and when were transferred to greenhouse they continued to grow.

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Poster Communication Abstract – 6A.39

PHENOTYPIC PLASTICITY AND QTL MAPPING OF BUD SET PROCESS IN *POPULUS NIGRA*

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Populus nigra, bud set, GxS interaction, phenotypic plasticity, QTL

The seasonal growth cycle of trees, such as bud break, growth, bud set and dormancy are distinct characters of perennial plants and represent a basic adaptations to their environment. The variability of the phenology of growth cessation and bud set correlates strongly with the latitude allowing the trees to find a compromise between the necessity to avoid the risk of frost damage and to maximize the length of the growing season. Even if photoperiod is widely accepted to be the main environmental signal for bud set in poplars, the timing of bud formation is also influenced by other factors such as temperature, temperature x photoperiod interaction, nutrition and drought. Research efforts to characterize the genetic basis of growth cessation and bud formation are of paramount importance to better understand the mechanisms at the basis of these traits. Besides, the role of phenotypic plasticity as a source of variability to determine short- and long-term plant response in different environment, can be used to evaluate the possibility of temperature-mediated plasticity in some genotypes more adapted to specific environmental conditions. A new protocol has been proposed recently to dissect the growth cessation in poplar and bud set key traits (phase, duration, sub-period) were defined to better characterize the bud set process. Data analysis has allowed to decompose the contribution of the different phenological traits to the dynamics of bud set in a *P. nigra* full-sib family (POP5) planted in two sites in central and northern Italy. Genetic variability, broad-sense heritability and phenotypic plasticity of these traits have been studied and QTLs analysis for the most discriminative traits has been performed using a multisite approach. Results showed that the onset of growth cessation is a quantitative trait under strong genetic control. Night length is the most important signal triggering the physiological process but the role of other environmental factors, such as temperature, increase during the process. Taking advantage of the two contrasting experimental sites a considerable role of GxS interaction has been found in all the different phenological phases and the low temperature seems to influence the sensitivity of some more plastic genotypes. QTLs identified in the POP5 genetic map, each one characterized by small

or modest effect, highlight the complex nature of the traits involved during the apical bud formation-maturation process.

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Poster Communication Abstract – 6A.40

DIFFERENTIAL SENSITIVITY OF ITALIAN RICE CULTIVARS TO SALT STRESS CONDITIONS

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Proline, oxidative stress, rice germplasm, salt stress tolerance, screening

Soil salinity is one of the main threat to agricultural production. FAO statistics report that nowadays more than 800 million hectares worldwide are affected by salt, about 6% of the earth's total surface, and the area is increasing. A soil is considered saline when ionic concentration exceeds an electrical conductivity of 4 dS m^{-1}, a value that corresponds to about 30-40 mM, depending on composition. Excess salt exerts both osmotic and ionic effects. Plants can cope with moderate salinity either by limiting uptake at the root level, by compartmentalizing/extruding ions in the vacuole/apoplast, or by counteracting the consequent water withdrawal through the intracellular accumulation of compatible osmolytes, the most common of which is the amino acid proline. At increasing soil conductivity, salinity stress causes a progressive reduction of the photosynthetic rate and stimulates the production of reactive oxygen species, which in turn leads to oxidative damages at the cellular level.

Rice (*Oryza sativa* L.), which represents a staple food for more than one third of world's population, is remarkably sensitive to excess salt, especially at the seedling level and at the flowering to panicle-initiation stage. A summary of field experiments pointed out that rice growth is even more sensitive than previously though, with a significant reduction of grain yield at an average seasonal salinity of the field water in excess of 1.9 dS m^{-1} (Grattan *et al*., Calif. Agric. 56:189-198, 2002). Because soil salinity has long been identified as a major issue for yield, many researchers investigated the occurrence of a differential sensitivity to salt stress among rice cultivars. However, all these studies dealt with Asian rice genotypes, whereas no information has been made available to date with respect to the Italian rice germplasm. Italy is the only country in Europe with a significant land area used for rice production, and the Italian rice germplasm comprises no less than 120-150 varieties belonging to the *japonica* ssp. Moreover, a valued rice is produced in the Northern Adriatic coastal region, that is vulnerable to salt inflow from the sea. This notwithstanding, the occurrence of a natural variability among these cultivars with respect to salt tolerance has never been investigated.

In the frame of a research project for integrated genetic and genomic approaches for new Italian rice breeding strategies, we aim at a better understanding of the biochemical bases for salt tolerance in rice. To achieve this goal, a few cultivars with a contrasting capability to cope with salt stress conditions will be identified, and the levels and the properties of selected enzymes playing a pivotal role in antioxidant defense and in proline metabolism will be studied.

Here we report the results of a preliminary investigation on the susceptibility to salt stress conditions of a panel of 11 Italian rice cultivars. The effect of salt (NaCl, $CaCl_2$, $MgSO_4$ and Na_2SO_4 in a 10:1:2:1 ratio) increasing concentrations, corresponding to a range of 0.7 to 12 dS m^{-1}, was investigated under axenic conditions either during seed germination or at the seedling level. Results showed the occurrence of a significant variability in salt sensitivity among varieties. A

differential tolerance was evident also with cell suspension cultures of the same genotypes, suggesting that it relies on biochemical processes expressed at the undifferentiated tissue level.

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Poster Communication Abstract – 6A.41

PHYTOTOXICITY OF PYRROLINE-5-CARBOXYLATE REDUCTASE INHIBITORS

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Aminobisphosphonates, amino acid inhibitors as herbicides, P5C, proline metabolism, programmed cell death under biotic stress conditions

Accumulation of high intracellular levels of proline has long been reported in many plant species under a variety of abiotic stress conditions. Unexpectedly, exogenously-supplied proline was found to exert phytotoxic effects and trigger the activation of programmed cell death (PCD). An early induction of the gene for δ^1-pyrroline-5-carboxylate (P5C) dehydrogenase, the enzyme that catalyses the second and last step in proline catabolism, was shown in several crops infected by virulent fungal strains. Moreover, proline accumulation through *de novo* synthesis has been reported in *Arabidopsis thaliana* during incompatible plant-pathogen interactions. Therefore the possibility exists that proline metabolism is involved in the process leading to PCD during the hypersensitive defence reaction. However, it is still unclear which may be the active molecule, whether proline itself or P5C, the intermediate in both its synthesis from, and its oxidation to glutamate. Some authors recently postulated that P5C might trespass the mitochondrial membrane, and that a P5C/Pro cycle would occur as a consequence, leading to reactive oxygen species production (Miller *et al*., J. Biol. Chem. 284, 26482–26492, 2009). This cycle would be mediated by the contrasting activity of proline dehydrogenase in the mitochondrion and P5C reductase in the cytosol.

The elucidation of these aspects has been hampered to date by the unavailability of p5cr$^-$ mutants. Proline can be synthesized from either glutamate or ornithine, but the two pathways share the last reaction, just catalyzed by P5C reductase. Therefore, in the absence of a functional enzyme, cells cannot synthesize proline. As a consequence, null mutations are embryo lethal. This also means that specific inhibitors of P5C reductase are expected to exert phytotoxic effects, and might represent new active principles for weed control. If available, they would represent as well useful tools to study the involvement of proline metabolism in the plant defence response against fungal pathogens.

We previously screened several aminomethylenebisphosphonates for their ability to inhibit *in vitro* P5C reductase. Some phenyl derivatives were indeed found to interfere in the micromolar range with the activity of the enzyme from *A. thaliana* (Forlani *et al*., J. Agric. Food Chem. 55, 4340-4347, 2007). Growth inhibition and reversal experiments performed with cell suspension cultures supported the possibility that P5CR inhibition does occur also *in vivo* (Forlani *et al*., J. Agric. Food Chem. 56, 3193–3199, 2008).

Here we report a troughout characterization of their effects at the whole plant level. The inhibitory potential of the most effective compounds, and of some new active analogues designed

on the basis of a structure-activity relationship analysis, was assessed on *Brassica napus* seedlings grown under axenic conditions. Data were compared with the inhibition brought about *in vitro* by the same substances on rapeseed P5C reductase, and related to the resulting steady-state levels of free amino acids in seedling tissues. Results showed phytotoxic effects in the micromolar to millimolar range, and confirmed the occurrence of proline starvation *in vivo*. Interestingly, significant variations in the homeostasis of other, unrelated amino acids were found, suggesting the existence of multiple targets of phosphonate action in plant amino acid metabolism.

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Poster Communication Abstract – 6A.42

CHARACTERIZATION OF POPLAR PLASTIDIC P2-G6PDH

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Plastidic glucose-6-phosphate dehydrogenase, G6PDH, OPPP, Populus trichocarpa

Glucose-6-phosphate dehydrogenase (G6PDH - EC 1.1.1.49) is a regulatory enzyme of the OPPP (oxidative pentose phosphate pathway) because of its tight regulation and/or redox dependence. The most important role of OPPP is to generate NADPH to provide reductants for biosyntheses. Reductants produced in the OPPP may also be important to fight oxidative stress (Wang *et al.,* 2003), induced by nutrient starvation, drought, salinity, pathogens. The Cy- and P2-G6PDHs are essential for stress tolerance (Esposito *et al.,* 2003; Nemoto and Sasakuma, 2000; Valderrama *et al.,* 2006; Wang *et al.,* 2008; Scharte *et al.,* 2009; Cardi *et al.,* 2011).

A fundamental role of redox regulation for the plastidial P2-G6PDH has been described, this isoform appearing strictly linked to the reductant balance within the plastids and to stress response (Esposito *et al.,* 2003).

Therefore, the gene encoding for the plastidic G6PDH isoform (P2-G6PDH) from poplar (*Populus trichocarpa*) has been cloned in the expression vector pET15b (His-Tag), and the recombinant protein overexpressed in *E. coli.* Moreover we generated single mutants of all cysteines and a double mutant for both cysteines presumably involved in the redox regulation.

The main kinetic parameters and the redox sensitivity to DTT and glutathione have been determined for all purified enzymes (wild type and all the mutants). The values measured for the wild type are in the same range as those obtained for most other P2-type G6PDHs (e.g. high Ki_{NADPH}). Additionally, the recombinant poplar WT enzyme is moderately sensitive to reductants (DTT_{red}), though it exhibits a redox potential (-280 mV) favourable for control by either thioredoxins *m* or *f*.

The study of the C175S and C183S variants confirmed that these cysteines are involved in the enzyme redox regulation. As these substitutions did not affect Km_{G6P}; this suggests that these mutations do not affect the active site, while the effects of NAPDH clearly indicate an non-competitive inhibition. On the other hand, the C145S and C242S variants did not display any activity suggesting an involvement in the tetrameric assembly of the enzyme. Regarding the C194S variant, the results suggest that this residue is not involved in the redox regulation since the substitution lefts Km_{G6P} substantially unchanged. Besides, this variant completely lost inhibition by NADPH, suggesting that C194 is part of the NADPH binding site.

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Poster Communication Abstract – 6A.43

THE GIBBERELLIC ACID RESPONSE OF DIFFERENT *VITIS VINIFERA* CULTIVARS DURING FRUIT SET

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Gibberellin, gibberellic acid, inflorescence, fruit set, Vitis vinifera

Bunch rot caused by mildews may lead to severe losses at harvest especially in the case of rainy seasons. For this reason, practices have been implemented to achieve berry thinning which allows to obtain aerated clusters also in cultivars with dense bunches that retain high humidity. The most common practice consists of treatments with gibberellins (GAs) at the moment of flowering (anthesis), which results in a reduction of fruit set and consequently produces looser bunches. GA treatments however have very different effects on different grapevine cultivars: the reduction of fruit set remains limited in the family of Pinot, where it is compensated by harvesting of healthier grapes, but results to be dramatic in other cultivars such as Sauvignon Blanc, with excessive yield's loss and effects persisting even in the years successive to the treatment.

Gibberellins (GAs) are plant hormones that regulate growth and influence various developmental processes, including germination, dormancy, stem elongation, flowering and fruit set. All known gibberellins are synthesized by the terpenoid pathway and then modified by several GA oxidases until they reach their biologically-active form. The two main active GAs synthesized in plants are GA1 and GA4, although it is yet not clear which form is mainly regulating developmental processes in grapevine inflorescences. Active GAs are eventually deactivated by modifications into inactive gibberellin forms. The pool of active gibberellins is maintained both by regulation of their biosynthetic pathway involving GA13, GA20 and GA3 oxidases, and their modifications through GA2 oxidases. Active gibberellins in plants are sensed by GID proteins (Gibberellin-insensitive-Dwarf), and the GA response is regulated mainly through a family of repressors of gene expression: the DELLA proteins. In the presence of the GA signal, GID interacts with DELLA proteins and direct them to proteolysis.

This work aims to characterize the molecular response to GAs of *Vitis vinifera* inflorescences from the cultivars Pinot Gris and Sauvignon Blanc, which show very different fruit set reduction in response to GA treatment.

We set up a analytical method to determine endogenous concentrations of several gibberellin species in grapevine inflorescences of the two cultivars, which allows us to detect up to nine different GAs starting from a methanolic extract separated by reverse phase UPLC chromatography coupled with a mass spectrometer.

In addition, this works aims to characterize the family of grapevine GA oxidases in terms of gene structure, gene expression and activity, and to determine whether their expression levels are different in the two cultivars following treatment with GA3.

Here, the most up-dated results of these investigations will be presented.

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Poster Communication Abstract – 6A.44

THE *MtTdp2* (5'-TYROSYL-DNA PHOSPHODIESTERASE) GENE IS INVOLVED IN THE PLANT RESPONSE TO GENOTOXIC STRESS

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DNA repair, Medicago truncatula, osmotic stress, photo-oxidative damage, *5'-Tyrosyl-DNA phosphodiesterase*

Because of their sessile lifestyle, plants are exposed to environmental stresses, that produce the highly cytotoxic Reactive Oxygen Species (ROS). To avoid ROS accumulation, plants have evolved complex antioxidant (enzymatic and non-enzymatic) mechanisms. Effective DNA repair pathways are activated by plant cells to remove oxidative DNA damage and preserve genome integrity [Balestrazzi *et al*. 2011,Macovei *et al*. 2011]. In animal cells, a novel DNA repair function encoded by the *TDP2* (5'-Tyrosyl-DNA Phosphodiesterase) gene has been recently described [Cortés-Ledesma *et al*. 2009]. The TDP2 enzyme is able to resolve the stabilized covalent complexes formed when DNA topoisomerase II (Topo II) binds DNA sites containing the oxidized nucleotide 8-oxo-deoxy-guanine (8-oxo-dG) or when cells are exposed to Topo II poisons [Zeng *et al*. 2011].The TDP2 function avoids the conversion of the stabilized DNA-Topo II complexes into double strand breaks (DSBs).

We report for the first time in plants on the *MtTDP2* gene identified and characterized in the model legume *Medicago truncatula.* Bioinformatic investigations carried out in plant databases have highlighted the presence of distinct TDP2 isoforms, which differ in the number of zinc finger RanBP2 domains. Quantitative Real Time PCR (qRT-PCR) analyses showed that *MtTDP2* is constitutively expressed in vegetative and reproductive tissues. *MtTDP2* is also significantly up-regulated by osmotic and photo-oxidative stress (provided by using the herbicide paraquat), suggesting a relevant role of the DNA repair response not only in the nuclear compartment but, possibly, in chloroplasts. Additional investigation on the gene expression profiles are currently in progress as well as the production of transgenic barrel medic lines overexpressing the *MtTdp2* gene.

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Poster Communication Abstract – 6A.46

A PROMISING ENERGY CROP FOR RURAL DEVELOPMENT: IMPROVEMENT OF *JATROPHA CURCAS* AGRO-PRACTICES

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Physic nut, *germplasm*, *intercropping, propagation, biodiesel*

Jatropha curcas L. is a valuable multipurpose crop and has recently gained lot of importance for the biodiesel production and secondary products such as soap, fertilisers, bio-pesticides, cosmetics, and medicine. The integration of *J. curcas* into rural economies in developing countries, through extensive plantations in marginal lands or intercropped agro-silvo-pastoral systems, could be an effective strategy to reduce local community dependence on imported energy resources, generate employment opportunities, and enhance their livelihoods.

J. curcas is a perennial drought-resistant plant well adapted to marginal lands in arid and semiarid and tropical regions. Furthermore, this energy crop offers the ecological advantage to mitigate soil degradation and desertification and to reclaim wasteland. Although the increasing global interest on this energy crop *J. curcas* physiological characteristics and its agronomic management practices are not thoroughly unravelled.

This contribution will highlight some *J. curcas* research challenges within the framework of an EU-AID international cooperation project implemented in Ghana. In order to improve the knowledge of *J. curcas* agronomical management practices, which could be transferred to the local Ghanaian rural communities, several agronomic experimental works are being carried out at Kwame Nkrumah University of Science and Technology's Agricultural Research Farm at Awomanso, (Kumasi, Ghana). Two-year-experiment outcomes on the evaluation of *J. curcas* local germplasms, of the most suitable generative propagation systems (direct seed *vs* pre-cultivated seedlings), and of *J. curcas* effects on intercropping system with *Arachis villosulicarpa* and *Zea mays* are discussed.

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Poster Communication Abstract – 6A.47

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF AMORPHA-4,11-DIENE SYNTHASE GENE IN *ARTEMISIA ANNUA* ANAMED A3

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Artemisia annua, artemisinin, amorpha-4,11diene synthase

The genus *Artemisia*, belonging to the Asteraceae family, contains a large number of aromatic plants. *Artemisia annua* is used in traditional Chinese medicine since more than 2,000 years. Recently this plant has received increasing attention because it produces the endoperoxide sesquiterpene lactone artemisinin (AN), which is widely used for the malaria treatment. Its synthesis has been achieved, but it is uneconomical and gives low yields due to complex chemical structure. Thus AN extracted from plant currently is only source of commercial AN drug. The plant produce relatively small amounts of AN and this has led to intense research in order to increase the yield of AN in the plant or to develop alternative methods of AN production.

In this work, we have studied the genomic organisation and expression level of amorpha-4,11-diene synthase gene (ads) in different genotypes of *A. annua* characterized by high level of AN (Artemis developed by Mediplant, Switzerland and Anamed A3 developed by Action for Natural Medicine) and compared results with those obtained from wild type plants. Amorpha-4,11-diene synthase (ADS) is a key enzyme in AN biosynthesis which catalyzes the first committed step, in which farnesyl diphosphate (FPP) is converted to amorpha-4,11-diene. A molecular probe has been developed based on sequences available in gene bank. This probe has been used to study ads gene copy number and its expression levels during flowering when glandular trichomes accumulating the sesquiterpene are formed.

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Poster Communication Abstract – 6A.48

CANDIDATE GENES CONTROLLING FRUIT QUALITY IN A TOMATO INTROGRESSION LINE TOLERANT TO WATER DEFICIT

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Solanum pennellii introgression lines (ILs), fruit quality, antioxidants, water deficit tolerance

Fruit quality is an important criterion for marketing of tomatoes and of paramount importance to guarantee consumer satisfaction. It involves a combination of many traits mostly expressing a quantitative variation and controlled by complex gene networks. In addition, fruit quality can be dramatically reduced by environmental constraints such as drought. Thus identifying genetic reservoirs of drought tolerance and elucidating genetic mechanisms controlling the interaction between drought stress and fruit quality traits may be beneficial for breeders to develop new tomato genotypes combining increased drought tolerance to high fruit quality. The aim of this research was to identify major genes and molecular networks controlling fruit quality under drought conditions in tomato.

Introgression lines (ILs), in which individual homozygous segments of wild chromosome are carried in the genomic context of the cultivated species, are useful for resolving complex traits in QTLs and identifying candidate genes. Screening ILs from *S. pennellii* allowed to identify IL 9-2-5 as a more drought tolerant genotype than the control M82. When grown at a lower water regime, IL9-2-5 also revealed to perform higher concentration of fruit reduced ascorbate (AsA). A comparative transcriptomic analysis allowed to select 62 transcripts differentially expressed in red ripe fruit of IL9-2-5 with respect to M82. Candidate genes for controlling fruit quality in tomato were identified among antioxidant pathways, signal transduction pathway, hormonal metabolism, transcription regulation process. In particular, the concentration of fruit AsA well correlated with the transcript abundance of cell wall-associated genes such as a polygalacturonase and a polygalacturanate-4-alpha-galacturonosyltransferase and with three monodehydroascorbate reductase genes.

Functional characterization of candidate genes will prove their involvement in fruit quality control and provide additional genetic means to breeders for tomato quality enhancement in sustainable cropping systems.