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Oral Communication Abstract – 1.01

REPETITIVE ELEMENTS TRANSCRIPTION AND MOBILIZATION CONTRIBUTE TO HUMAN SKELETAL MUSCLE DIFFERENTIATION AND DUCHENNE MUSCULAR DYSTROPHY PROGRESSION

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Noncoding RNAs (ncRNAs) are recently considered component of chromatin, having a critical role in organizing the epigenome architecture and epigenetic memory. Genome-wide studies have revealed that ncRNAs transcription, mostly originating within intergenic regions of the genome, is far more ubiquitous than previously thought. A large part of thee transcripts originate from repetitive sequences. To this, we recently reported the first complete transcriptome produced by repetitive elements in the mammalian genome (Faulkner et al, Nat Genet 2009), which covers about 20% of overall transcripts in a cell. This study revealed that repetitive element expression is regulated in a tissue specific manner and that their expression is positively correlated with expression of neighboring genes. Notably, LINE signal dependent expression appears to be linked to their genomic redistribution, as recent reports showed de novo LINE-1 (L1) retrotransposition events in somatic as well as cancer cells (Coufal et al., Nat 2009; Huang et al., Beck et al, Iskow et al. Cell 2010). It as also been shown that L1 retrotransposition can be controlled in a tissue-specific manner and that disease-related genetic mutations can influence the frequency of L1 retrotransposition (Muotri et al Nat 2010). These findings suggest a potential role of mobile elements as mediators of somatic variations, which in turn can influence the genome and the epigenome plasticity in order to accomplish developmental programs.

The role of noncoding transcriptome in skeletal muscle cell differentiation is unexplored and it may represent an opportunity to unravel and characterize its contribution to dystrophic muscle degeneration.

To this we generated deepseq transcriptome CAGE libraries from three Duchenne Muscular Dystrophy (DMD) patients and three controls' primary myoblasts. Cytosolic and nuclear RNA fractions were collected and deep-sequenced at different time points: proliferating myoblasts, myotubes upon differentiation induction (day 1 of differentiation) and differentiated myotubes (day 8 of differentiation). This analysis highlighted that LINEs constitute the bulk of repetitive element transcription and that the resulting RNAs are selectively localized in the nucleus. Notably the largest difference between DMD and control samples appears to be in nuclear transcriptome of all repetitive elements including LINE-1. Further, by using a Taqman-based approach, we analysed L1 copy number variation in proliferating and differentiating myoblasts derived from the same DMD patients and healthy donors; surprisingly, new retrotrasposition events occured during control's differentiation and not during DMD's differentiation and profound differences are featured between patients compared to control.

Data will be presented showing a direct link between L1 transcription, myogenic program and its alteration in DMD progression.

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Oral Communication Abstract – 1.02

ENVIRONMENTAL EPIGENETICS IN MAIZE: ADVANCES FROM A EUROPEAN INITIATIVE

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Epigenetics, Zea mays, abiotic stress, transposable elements, chromatin

AENEAS (Acquired Environmental Epigenetics Advances: from Arabidopsis to maize) is a research collaborative project recently funded by the European Commission. The AENEAS initiative aims to assess the impact of environmental conditions on epigenetic states in the model plant *Arabidopsis thaliana* and then transfer knowledge to maize, one of the most important crops of the world. Here, we illustrate some of the recent advances obtained by studying maize epigenetics within AENEAS project.

A first objective of AENEAS is to characterize three epi-regulatory pathways in maize. These pathways are: the autonomous flowering (AF), the CpG methylation (mCG), and the small RNA (sRNA) pathway. These pathways are well characterized in Arabidopsis, particularly for their interaction with environmental signals in mediating changes into the epigenome. The expected outcome of our study is the production of maize tools (e.g. mutants and epi-targets) for analyzing epigenetic-mediated response to environmental cues. In this context, we have focused our study on the maize homolog of the Arabidopsis *FVE* gene, named *nfc102*, which encodes a MSI-like WD-repeat protein belonging to AF pathway. The *nfc102* gene is ubiquitously expressed, but its RNA accumulates in actively dividing tissues and *nfc102* antisense transcripts were detected in specific tissues. Phenotypic analysis of *nfc102* RNAi mutants reveals that plants exhibit several developmental defects, suggesting a pleiotropic *nfc102* function. Among sequences showing differences in RNA accumulation in *nfc102* mutant compared to wild-type plants is enclosed the maize homolog of the Arabidopsis florigen *FT* gene (*ZCN8*). Our results suggest that *nfc102* is involved in the regulation of *ZCN8* sense and antisense RNA processing. Additional targets of *nfc102* are different types of transposons and retrotransposons (TEs), which show an increase of their RNA level in *nfc102* mutants. The *nfc102* down-regulation also provokes in the targeted TEs a decrease of histone marks associated with transcription activation and an increase of histone modifications related to silencing, thus corroborating the *nfc102* role in epigenetic-mediated silencing.

Since *nfc102* regulates the RNA level of various TEs and because it is well known that TEs are sensitive to different environmental cues, we have performed experiments to analyze TEs response in *nfc102* mutants concomitantly with application of temperature stresses. In these experiments we have enclosed the *rmr6* mutant, affecting the function of the maize homolog of the

Arabidopsis *NRPD1* gene, which encodes the largest subunit of RNA polymerase IV. Since retrotransposition occurs through retrotranscription of TE-derived RNAs, the level of both transcripts and extrachromosomal DNA copies for the TEs was detected. The analysis was performed immediately after stress removal and after some days of recovery to assess maintenance of changes through mitotic division. Preliminary results from these experiments will be illustrated and discussed.

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Oral Communication Abstract – 1.03

DETERMINING THE CORRELATION BETWEEN METHYLATION AND GENE EXPRESSION AT A FLOWERING TIME QTL IN MAIZE

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Flowering time, cytosine methylation, Zea mays

In maize, the genetic control of flowering time has been investigated in several quantitative trait locus (QTL) studies. One of the major QTLs for flowering time, the *Vegetative to generative transition 1* (*Vgt1*) locus, corresponds to an upstream (70 kb) non-coding regulatory element of *ZmRap2.7*, a repressor of flowering. Among the polymorphisms that distinguish late and early *Vgt1* alleles, the insertion of a MITE transposon was found to be highly associated with flowering time in a number of independent studies. As cytosine methylation is known to influence gene expression, we aimed to determine if methylation might be involved in the relationship existing between *Vgt1* and *Rap2.7*. The methylation state at *Vgt1* was assayed using an approach that combines digestion with McrBc (an endonuclease that acts upon methylated DNA), and quantitative PCR. The analyses were performed on genomic DNA from leaves of six different maize lines at four stages of development. The results showed a trend of reduction of methylation from the first to the last stage with the exception of a short genomic region flanking the MITE insertion, where both alleles display a stable and very dense methylation throughout leaf development. Bisulfite sequencing of a small portion of *Vgt1* revealed differential methylation for a single cytosine between the two alleles. Finally, *ZmRap2.7* expression was determined at four developmental stages for the six genotypes and found to correlate with *Vgt1* methylation.

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Oral Communication Abstract – 1.04

THE EPIGENETIC REGULATION OF CENTROMERE AND CHROMOSOME SEGREGATION

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Epigenetic regulation, SAGA complex, centromere, kinetochore

The centromere is a chromosome region defined by a variant nucleosome needed to prevent chromosome loss and aneuploidy. Loss of HAT Gcn5 causes several mitotic defects and chromosome loss. We are studying the consequences of an aberrant epigenetic regulation caused by deletion of key epigenetic regulators on mitotic progression in budding yeast.

We show that deletion of SAGA components HAT-Gcn5 and DUB-Ubp8 leads toan aberrant association of histone variant at the centromere in yeast. This and other results clearly indicate an interesting epigenetic cross-talk which regulate the main organelles controlling chromosome segregation and Mitosis.

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Oral Communication Abstract – 1.05

MITOCHONDRIAL DNA VARIABILITY INFLUENCE GLOBAL DNA METHYLATION LEVELS

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mtDNA variability, global DNA methylation, cybrid cell lines, MAT1A gene, ROS

Epigenetic changes to DNA during human lifetime are modulated by environmental and genetic factors. Among these factors, mitochondrial functions are emerging to have a key role, probably to their central position between energy uptake and energy production.

Mitochondrial DNA inherited variants (mtDNA), determining mtDNA haplotypes and groups of haplotypes (haplogroups), are thought to contribute to the inter-individual variability in mitochondrial function. These variants affect the quality of aging and age-related pathologies, such as Alzheimer's and Parkinson's diseases, diabetes and cancer. The molecular mechanisms by which mtDNA variability influences these traits are likely to involve a complex interaction between mtDNA and the nuclear genome, possibly through the modulation of oxidative phosphorylation (OXPHOS). The importance of this interaction has also emerged either from studies on epigenetic changes and, more specifically, on the DNA methylation of cytosines, either from *in vitro* studies carried out in cybrids, engineered cells that share the same nuclear genome but harbor different mitochondrial genomes.

On the basis of these observations, population and *in vitro* studies were carried out to investigate the relationship between age-related epigenetic modifications and mtDNA variability. To this purpose, using methyl-sensitive restriction endonucleases (*CpG*lobal method), we measured global DNA methylation levels both in peripheral blood DNAs collected from 354 (153 males and 191 females) unrelated adult subjects, previously analyzed for their mtDNA variability, and in cybrid cells harbouring mtDNA molecules of H, J, U, X, and T haplogroup. In these cells we also analyzed the expression profiles of different genes involved in methylation processes and the ATP and ROS levels, important regulators of the above processes and key elements of the mitochondria-to-nucleus cross-talk.

From our population association study and *in vitro* analyses, it has emerged that the subjects and cybrid cells harboring mtDNA molecules belonging to the J haplogroup have higher global DNA methylation levels than non-J carriers. Moreover, in this cell line we measured an over-expression of the methyltransferase *MAT1A* gene and low ATP and ROS levels.

Data we obtained indicate that mtDNA variability could influence DNA methylation by regulating the expression of MAT1A gene that plays a crucial role in these processes. We hypothesize that this influence is likely exerted through the activation of mediators of the cross-talk between mitochondria and nucleus such as ATP and ROS. In fact low ATP and ROS levels occurring in the J cybrids might induce the activation of transcriptional activators of the *MAT1A* gene, leading to its over-expression and thus to DNA hypermethylation.

On the whole our data provide the first evidence that mtDNA variability modulates global DNA methylation levels, possibly via the regulation of OXPHOS efficiency and indicate that

mtDNA-specific interactions between mitochondria and nucleus could regulate epigenetic changes in a mtDNA haplogroup dependent-manner.

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Oral Communication Abstract – 1.06

ISOLATION OF microRNAs FROM ARTICHOKE AND THEIR INVOLVEMENT IN THE REGULATION OF TARGET GENES

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Artichoke, deep sequencing, microRNA, salt stress

MicroRNAs (miRNAs) are the products of endogenous non-protein-coding genes, originated from hairpin-like single stranded RNA precursors (pre-miRNAs and pri-miRNAs). miRNAs have been shown to affect gene expression at the post-transcriptional level through mRNA cleavage or translational repression. Recently, a new class of miRNAs (23–27nt long), have been demonstrated to guide cytosine DNA methylation both at their own gene loci in cis, and at their target gene loci in trans, possibly resulting in transcriptional gene silencing.

In order to characterize artichoke miRNAs, the present, we sequenced sRNA libraries obtained from artichoke leaves and roots using Illumina technology. Libraries were obtained from control tissues, and from the same tissues after saline stress treatment.

Artichoke sRNA fraction included mainly sequences in the range of 16-30 nucleotides in length, with a peak at 24 nucleotides. Bioinformatic analyses allowed to identify 83 artichoke conserved miRNAs, belonging to 25 families, and a number of novel species-specific artichoke miRNAs.

Differential expression of miRNAs was evaluated by normalizing the abundance of each miRNA in sRNA libraries as transcripts per million, and comparing stressed with control tissues, and by means of real-time PCR.

Potential miRNA precursors were identified by BLASTn searches of the mature miRNA sequences against artichoke ESTs and newly obtained Illumina genomic sequences and fold-back structure was predicted.

A bioinformatic search of miRNA targets produced over 40 putative target sequences, homologous to characterized *Arabidopsis* proteins. Most targets were transcription factors, and some of them had already proved to be involved in stress response in other plants.

Experimental validation of targets provided evidence on the mechanisms of action of artichoke miRNAs.

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Oral Communication Abstract – 1.07

EPIGENETIC CONTROL OF RNA POLYMERASE II TRANSCRIPTION AND DNA RECOMBINATION BY H4K16 ACETYLATION, AT rDNA OF *S. CEREVISIAE*

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S. cerevisiae, histone acetylation, Sir2, aging, epigenetics

In *S. cerevisiae* the ribosomal locus (rDNA) represents a region in which DNA replication, transcription and recombination meet both physically and functionally. The locus is a single gene cluster, consisting of approximately 150 repeated units. Each repeat contains the 35S and 5S genes, transcribed by RNA Polymerase I and III, and a non transcribed spacer (NTS). Despite its name, the latter sequence could be transcribed by RNA polymerase II to produce non coding RNAs (ncRNAs). In yeast, ribosomal ncRNAs transcription, may locally displace cohesins leading to rDNA copy number variation and extrachromosomal ribosomal circles (ERCs) production. These events are considered markers of genome instability and aging. Recently it has been shown that also the replication efficiency is essential for rDNA recombination rate. Each unit contains an ARS element (rARS) but only 20% of these origins are active in a single cell cycle. Once a rARS has fired, replication proceeds bidirectionally but the leftward-moving fork is blocked at the RFB (replication fork barrier) site. DSBs originating at the stalled forks may lead to ERCs formation and copy number variation. All this implies that the basic DNA transactions, DNA replication, Recombination and Pol I, II and III transcription, occurring at rDNA need to be finely controlled.

Interestingly, mutant strains of the NAD-dependent histone deacetylase Sir2p increase their replication efficiency, recombination rate and ncRNA production, suggesting that these processes are epigenetically linked. Considering the NAD-dependent histone deacetylase activity of Sir2p we want to investigate whether other sirtuins of *S. cerevisiae* (*HST*1-4) could affect recombination and/or RNA Pol II transcription and/or DNA replication, and to verify if the relationship is based on histone acetylation.

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Poster Communication Abstract – 1.08

A MULTIPLE APPROACH TO STUDY ENVIRONMENTAL STRESS-INDUCED EPIALLELE FORMATION AND INHERITANCE IN *ZEA MAYS*

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DNA methylation, environmental stresses, epigenetic marks, Zea mays

In the last years it has became evident that epigenetic marks such as DNA methylation, histone modifications, histone variants and small RNA populations play a primary role in creating alternative states of gene expression. Some marks define different epigenetic reversible states in responses to environmental or/and developmental inputs and thus will not have any evolutionary impact. On the contrary, the formation of epialleles that can be propagated mitotically and meiotically transmitted to the progeny, remaining stable for several generations, could play an important role in plant adaptation and evolution. In particular, environmental cues activate specific epigenetic mechanisms, which add epigenetic marks, altering patterns of gene expression, destabilizing the plant genome and causing phenotypic changes. Thus, environmentally triggered formation of epialleles and their transmission represent an important, yet unexplored, source of variation and adaptive power that can contribute to improvement of crop plants.

The FP7 European project entitled AENEAS (Acquired Environmental Epigenetics Advances: from Arabidopsis to maize) aims to "explore" environmentally-induced epigenetic changes as the "new frontier" of natural and artificial variability. In this framework we are investigating the m$echanisms of environment-induced epiallele formation and their heritable maintenance in maize. More in details, we are analyzing at genome-wide level the effects of cold stress on DNA methylation profiles coupling bisulfite conversion of unmethylated cytosines with Illumina sequencing (BIS-Seq). Preliminary results from this analysis will be presented and integrated with the data on epigenetic gene expression regulation in response to cold stress obtained *via* mRNA-, miRNA- and CHIP-Seq by other groups participating in AENEAS project. These results obtained by different approaches will allow to identify a robust list of sequences target of epigenetic regulation (epitargets) belonging to three main epigenetic pathways (autonomous, small RNA and CpG methylation). In this context we are also characterizing several epiregulators belonging to three pathways by gene expression analysis and mutant production. In this first step we focused our attention on *NFC102*, the maize ortholog of the Arabidopsis *FVE* gene, which encodes a MSI-like WD-repeat protein belonging to autonomous flowering pathway. Expression analysis revealed a complex expression pattern in young and actively dividing tissues and an antisense transcript has been detected as well.

Finally, reproducible protocols for temperature shift treatments, salinity and drought stresses have been optimized to induce and investigate epigenetic changes in maize, validating the previously identified epitargets and analyzing their trans-generational inheritance in stressed wt and

mutant plants. Since the induction of alternative epigenetic states not only triggers the formation of novel epialleles but also promotes the movement of DNA transposons and retroelements that are very abundant in plant genomes, we are investigating the stress effect on transposons mobility.

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Poster Communication Abstract – 1.09

DNA METHYLATION ANALYSIS IN RAPESEED (*BRASSICA NAPUS* VAR. *OLEIFERA* DEL.) UNDER SALT STRESS BASED ON M-SAP MARKERS

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DNA-methylation, M-SAP, salinity, germination, growth

Salinity is an important limiting environmental factor for rapeseed production worldwide and can hamper initial developmental stages in Mediterranean climates where the crop is sown in late summer. DNA methylation is known to play a crucial role in regulating plant development and tissue differentiation. In this study, we compared the extent and pattern of cytosine methylation in one tolerant and one sensitive rapeseed (*Brassica napus*) cultivar, germinated in distilled water and grown either in distilled water or in a 150 mM NaCl solution, using the technique of methylation-sensitive amplified polymorphism (M-SAP). Analysis of amplification products generated by eleven primer combinations showed that the rapeseed genome is hypermethylated with several polymorphic fragments. In particular, under salt stress conditions, the tolerant cultivar showed more DNA methylations than the stress sensitive one.

Forty-six methylation-related fragments were recovered from tolerant and sensitive cultivars, cloned, sequenced and subjected to BLAST analysis. Eight sequences shared high homology with *Arabidopsis thaliana* genes somehow related to stress tolerance: trehalose phosphatase/synthase, LEUNIG, SH3 domain-containing proteins, radical SAM domain, fringe-related protein, glutamine fructose 6 phosphate, CYP86A8 and DNA methyltransferase.

Validation of results through the analysis of tissue-specific gene expression using real-time PCR is reported and discussed.

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Poster Communication Abstract – 1.10

METHYLATION PATTERNS IN *BRASSICA OLERACEA* L.: CHANGES DURING TIME AND BETWEEN DIFFERENT AGROCLIMATIC CONDITIONS

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Brassica oleracea, methylation, M-SAP technique

Among the *Strategies for Organic and Low-input Integrated Breeding and Management* (SOLIBAM) project aims, there is the identification of epigenetic modifications caused by farming under different agronomic and/or pedoclimatic conditions. SOLIBAM project is funded by the European Commission under the Seventh Framework Programme (GA 245058, coord. Véronique Chable, INRA, scientific responsible Valeria Negri, UNIPG).

Plants of two hybrid varieties of broccoli (*Brassica oleracea* L. *var. italica* Plenck), Iron Man (IM) and Santee (SN) were grown in three experimental fields under different climatic and management conditions: low input (LI) and organic agriculture (OA). Young and healthy leaves were collected from both varieties and from each experimental field at three different dates during winter time. Differences in methylation state were identified applying the *Methylation-Sensitive Amplified Polymorphism* technique (M-SAP) using 5 different primer combinations on genomic DNA extracted from 36 samples (2 plants for each of the 2 hybrids, grown in 3 different experimental fields and collected at 3 different times).

From a total of 210 produced amplicons (with an average of 42 for each primer combinations) 25 were specific of IM, 13 of SN and 172 were found in both hybrids. Seventy two amplicons in Iron Man (36,5%) and 70 amplicons in Santee (37,8%) were from non-methylated regions. Among the amplicons from methylated regions, 41 (21,4%) in IM and 51 in SN (27,6%) showed the same profile at the three growing stages. Presence *vs* absence of 83 amplicons in IM and 64 in SN underlined changes in methylation patterns in relationship to different sampling dates, while 17 in Iron Man and 19 in Santee are putatively related to the different agro climatic growing conditions. Intriguing enough, 9 of these amplicons are common between the two analyzed hybrids. Changes in methylation state have generally occurred from the non-methylate or the hypermethylated pattern to the intermediate states or *vice versa*. Direct shifts between hyper-methylated to non-methylated state were rarely recorded.

Considered the large number of polymorphic amplicons obtained, the M-SAP technique appears to be an appropriate method to identify the methylation state modification. The technique will be used in further studies aimed at assessing broccoli epigenetic modifications due to different climatic and management conditions.

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Poster Communication Abstract – 1.11

CITRUS TRISTEZA VIRUS RESISTANCE GENE LOCUS: SMALL RNA PROFILE AND PRELIMINARY EPIGENETIC STUDIES

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Resistance gene, tristeza, siRNA, DNA methylation

Small interfering RNAs (siRNAs), play a vital role in epigenetics of plant virus-host plant interactions. It has been extensively studied at both the transcriptional and post-transcriptional levels. In plants, siRNAs initiate and manage gene silencing by directing DNA methylation and/or histone methylation. In Arabidopsis, the ~24 nt siRNAs directs DNA methylation (RNA-directed DNA methylation, RdDM) and chromatin remodeling at their target loci. Recent advances in high-throughput sequencing techniques has enabled thorough exploration of small RNAs populations and allow rapid analysis of massive datasets to assemble complete full-length genome sequence for different plant species. This large database of sequence information also allows identification of genome regions specifically matched by siRNAs that likely differ among tolerant, resistant or susceptible hosts and advance epigenetic studies on diseased plants.

Resistance to *Citrus tristeza virus* (CTV), the most severe virus affecting *Citrus* spp., associated with a single dominant gene locus *Ctv* occurring in *Poncirus trifoliata* while all *Citrus* spp. are considered susceptible. This locus contains 22 putative genes, but their regulation and mechanism for resistance remains unknown.

In our study, CTV was graft-inoculated on Carrizo citrange (*Poncirus trifoliata* x *C. sinensis (I think)*) and *C. aurantium* (sour orange) seedlings, and the population of siRNA characterized by high-throughput sequencing using an ILLUMINA platform. The *Ctv*-derived siRNA (~2% of the total short reads) were dominated in both hosts by the 24-nt. However, CTV infection caused an increase in accumulation of 24-nt siRNA sequences homologous to the *Ctv* gene in Carrizo but it decreased in sour orange. Distribution of the 24nt along the *Ctv* gene locus (282Kb) had a clearly different distribution between the two host. The predominant hot spot of siRNA in Carrizo mapped in the putative gene *Ctv-20*, whereas in sour orange it associated to the intergenic region between the putative genes *Ctv-11* and *Ctv-12*, where a Copia-like retrotransposon C is located. This distribution profile was conserved for each species between CTV-infected and uninfected plants but, as previously mentioned, the frequency of the 24nt siRNAs was altered by the presence of the virus.

We supposed that the different profile of 24nt between the two host in the locus *ctv* is due to RdDM mechanisms. To demonstrate the methylation status of the resistance locus we performed a bisulfite treatment of DNA. in which unmethylated cytosine was converted to uracile, while methylated cytosine did not react. A methylcytosines mapping was carried out on *Ctv-11* and *Ctv-12* sequences. By specific software were found 5 different CpG islands in the Copia-like

retrotransposon sequence and 42 primer pair were designed. The PCR analyses have been carried out using MSP and BSP primers followed by combined bisulfite restriction analysis (COBRA).

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Poster Communication Abstract – 1.12

DOWN-REGULATION OF A HISTONE DEACETYLASE AFFECTS MALE MEIOSIS IN *ARABIDOPSIS*

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Arabidopsis thaliana, histone acetylation, microsporogenesis

Recent studies showed that histone post-translational modifications (HPTMs) are associated to meiotic events including homologous recombination, cohesion, chromosome segregation. Our previous work in *Arabidopsis* evidenced that histone acetylation is required for meiotic recombination and chromosome segregation in male meiosis (*Perrella et al. Plant J 2010, 62: 796*). Histone acetylation is a reversible process carried out by two classes of enzymes known as histone acetylases (HATs) and histone deacetylases (HDACs). In this work, we focused our attention on a HDAC recognized as a global regulator that is involved in different physiological and developmental processes in *Arabidopsis*. Down-regulation of this HDAC mediated by antisense RNA and T-DNA insertion caused male and female sterility. In order to elucidate the mechanisms underlying the reduction of plant fertility the functional role of this HDAC was investigated in male meiosis in T-DNA mutant characterized by lower expression level and transcript rearrangement as compared to wild type. Different abnormalities affecting pairing, recombination and chromosome segregation have been observed in the mutant. As compared to wild type meiocytes, homologous chromosomes appeared not fully synapsed in pachytene. At diplotene/diakinesis, two univalents were observed in 17% of male meiocytes indicating a failure of the obligate CO for one chromosome pair. At later stages, uneven chromosome distribution was evidenced, as well. It is likely that the meiotic defects are depending on the histone hyperacetylation caused by HDAC down-regulation.

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Poster Communication Abstract – 1.13

A HISTONE DEACETYLASE IS REQUIRED FOR FERTILITY, SEED GERMINATION AND SEEDLING GROWTH RATE IN *ARABIDOPSIS*

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Arabidopsis, epigenetics, reproduction

Histone post-translational modifications (HPTMs) play a fundamental role in many aspects of plant development and interaction with environmental stimuli. In *Arabidopsis*, several works underlined the involvement of histone de-/acetylation in the determination of flowering time, organ identity, flower morphology and fertility. Based on *in silico* analysis which looked for histone acetylases/deacetylases (HATs/HDACs) preferentially expressed in *Arabidopsis* flower buds and orthologous genes involved in sexual reproduction in other organisms, a HDAC (hereinafter named *HDAC1*) was identified as the strongest candidate for a reproduction role in *Arabidopsis*. In this work, the function of this gene was investigated by reverse genetics. Plants over-expressing *HDAC1* (hereinafter oe*HDAC1*) as well as lines silenced by artificial miRNA (hereinafter ami*HDAC1*) have been analyzed. Both oe*HDAC1* and ami*HDAC1* show a drop in seed germination and changes of seedling growth rate. Moreover, ami*HDAC1* plants exhibit additional defects affecting plant reproduction. Indeed, delayed embryo development, seed abortion and silique semisterility were observed as well as abnormal ovules and defects in bivalent disjunction during microsporogenesis. Further molecular, biochemical and cytological analyses are being carried out to investigate the role of HDAC1 in *Arabidopsis* reproduction.

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Poster Communication Abstract – 1.14

THE MAIZE WD-REPEAT CHROMATIN REMODELING GENE *nfc102* REGULATES THE MAIZE HOMOLOG OF THE *ARABIDOPSIS* FLORIGEN *FT*

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Epigenetics, Zea mays, chromatin remodelling, flowering, sense and antisense RNA

The *nfc102* gene encodes a WD-repeat protein belonging to the *Multicopy suppressor of IRA* (*MSI*) family, originally identified in yeast. In maize, five genes of the *MSI* family have been identified and named *nfc*, because they display homology with one of the NURF complex component, where NURF is the Nucleosome Remodeling Factor: a multi-proteins complex that regulates transcription by catalyzing nucleosome sliding. Among the members of the *MSI* family, the maize *nfc102* gene displays high sequence homology with the Arabidopsis *FVE*, which is a component of the autonomous flowering pathway and that regulates transposons transcription. Although *FVE* is known as a positive flowering regulator, mainly because it inhibits expression of the flowering repressor locus *FLC*, it has been recently shown that *FVE* can also negatively affect transcription of the Arabidopsis florigen *FT*. In all cases, *FVE* acts by affecting histone modification pattern, thus provoking changes in the chromatin structure of its targets.

In maize, none of the several members of the MADS-box transcription factor class identified so far exhibits a functional homology with the Arabidopsis *FLC*. However, the maize homolog of the Arabidopsis *FT* gene has been recently identified and named *ZCN8*. *FT* orthologs from different plant species were shown to behave as a flower-forming signal because they were transcribed and translated in leaves, but their proteins subsequently move through the phloem to the SAM, where they induced the floral transition upon reaching a critical concentration. Interestingly, *ZCN8* transcript is detected almost exclusively in an unspliced form in tissues enriched for meristematic area (MA), while the spliced transcript form is produced only in the leaf blades (LB). Using strand specific RT-PCR we observed that the unspliced *ZCN8* RNA transcript is mainly represented by the antisense *ZCN8* RNA strand, which was named ZAS1. A shorter antisense *ZCN8* RNA strand, named ZAS2, was also detected, corresponding to RNA with alternative splicing with respect to the spliced sense RNA strand. The *ZCN8* sense RNA strand was almost exclusively present in LB as a fully spliced form, with an ORF that encodes for the ZCN8 protein.

Analysis of the *ZCN8* sense and antisense RNA levels in *nfc102* RNAi mutant compared to wild-type plants showed that *nfc102* down-regulation affect the level of both *ZCN8* sense and antisense transcripts and the ratio between the amount of ZAS1 and ZAS2. In this poster we will illustrate in details these findings and we will discuss about the possible role of *nfc102* in controlling *ZCN8* expression by linking changes in chromatin structure with RNA processing.

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Poster Communication Abstract – 1.15

BIOCHEMICAL AND PHYSIOLOGICAL EFFECTS OF *PSBS* GENE SILENCING BY RNAi IN *SOLANUM LYCOPERSICUM*

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Gene silencing, plastids differentiation, Solanaceae, photoprotection, heat dissipation

Photosynthetic organisms convert light energy into chemical energy used in order to produce biomass. This special feature has been widely exploited by human beings for edible purposes, harvesting leaf tissues, seeds or fruits of several species, now intensively cultivated. Photosynthesis, however, brings as a side product the production of Reactive Oxygen Species (ROS) that can damage cell membranes (photoinhibition), especially when the light energy available is in excess, saturating the photosynthetic machinery. One of the major mechanisms developed by higher plants in order to reduce photoinhibition is the thermal dissipation of light energy absorbed by chlorophylls preventing energy transfer to oxygen and thus ROS formation. This mechanism, named Non-Photochemical Quenching (NPQ), in higher plants is strictly dependent on the presence of a Lhc (Light Harvesting Complex) -like protein, PsbS, which differently from other Lhc proteins does not bind pigments, but stimulates NPQ interacting with Photosystem II antenna proteins. This mechanism is very efficient, inducing thermal dissipation up to 80% of absorbed light even at moderate irradiance, suggesting that evolution lead higher plants to fully protect themselves at the expense of partial a loss of light energy conversion efficiency. Preliminary analysis conducted on an *Arabidopsis thaliana* mutant missing PsbS protein (npq4 mutant) indeed showed an increased growth at low light as compared to WT, suggesting that the deletion of this photoprotective mechanism might have an industrial application for plants cultivated in control conditions as in greenhouses, where the light can be artificially modulated. For example, the npq4 phenotype could be used to reduce the light energy needed for obtaining the same amount of biomass for tomato plants grown in greenhouses in northern countries, thus reducing the cost of cultivation. In this work, we produced different lines of *Solanum lycopersicum* transformed with a construct for RNA interference (RNAi) against the *psbS* gene. Here we report the physiological and biochemical characterization of two of these lines, one with complete absence of PsbS proteins, and one with a 30% protein left. Possibilities of industrial application of this mutation are discussed.

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Poster Communication Abstract – 1.16

THE YETI GENE OF *DROSOPHILA* ENCODES A BCNT PROTEIN REQUIRED FOR CHROMOSOME ORGANIZATION

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Recent evidence indicate that the presence of transcribed genes in constitutive heterochromatin is a conserved trait that accompanies the evolution of eukaryotic genomes. Thus, the notion that heterochromatin is merely a synonymous of gene silencing has become obsolete. Our research is aimed at studying the functional aspects of constitutive heterochromatin in *Drosophila melanogaster*, by combining classical genetic and functional genomic approaches. One of the goals of our work is to explore the functions of the vital genes located in this enigmatic portion of the genome. In the present work, we have performed a functional analysis of *YETI*, a heterochromatin gene that encode a protein belonging to the evolutionarily conserved BCNT family, whose function is still unclear. We found that YETI binds to polytene chromosomes and, possibly via interactions with TIP60 chromatin remodeling complex, is responsible for proper deposition of H2Av on chromosomes. Moreover, our findings strongly suggest a conserved role in chromosome organization of the YETI protein and its human ortholog, CFDP1, which is extremely interesting in the light of the involvement of CFDP1 in complex developmental syndromes. Our study shed light on the function of YETI and CFDP1 proteins and may serve as unifying example of the key role played by the BCNT protein family in chromosome organization.