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Oral Communication Abstract – 8.01

FRUCTANS COUNTERACT ROS IN PLANTS, IN FOOD AND IN THE HUMAN BODY

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Fructan, prebiotics, antioxidant, ROS, food, disease, longevity

Fructans are fructose-based oligo- and polysaccharides accumulating in the vacuoles of a number of plant species (e.g. wheat, chicory). Since long, they are recognized as water-soluble reserve compounds that can be quickly remobilized to sustain growth. They are also believed to increase plant stress tolerance, and act as osmoregulators and sink strength regulators. Fructans are also gaining importance as prebiotics to improve health. Recent data on transgenic plants carrying fructan- and other sugar biosynthetic enzymes strongly suggest that sugars can also act as scavengers of reactive oxygen species (ROS) *in planta*. This antioxidant activity is confirmed by *in vitro* measurements. Models are presented to explain how vacuolar antioxidant mechanisms might cooperate with the more classic, cytosolic antioxidative defence mechanisms. The dual character of fructans, acting both as prebiotics and as antioxidants, make them even more promising for future use in functional foods. Their antioxidant properties are highlighted along the gastrointestinal tract. Novel insights strongly suggest that they might not only contribute to general health and well-being, but likely they also counteract ROS-based diseases (e.g. cancer), prevent outbreak of pathogens (e.g. *Salmonella*) and, in combination with restricted caloric intakes, they might even contribute to longevity.

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Oral Communication Abstract – 8.02

CANTHAXANTHIN PRODUCTION BY *SCENEDESMUS* SP., A CHLOROPHYTA FROM ANTARCTICA

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Scenedesmus sp., light and temperature changes, pigments, ultrastructure

Scenedesmus sp. is a coccoid Chlorophyta, isolated from a lake near the Gondwana Station (Antarctica). Cultures of this photosynthetic microorganism were grown at three different conditions: 10 µmol photons m^{-2} s^{-1} at 4°C (low light and temperature); 100 µmol photons m^{-2} s^{-1} al 4°C (high light and low temperature) and 10 µmol photons m^{-2} s^{-1} at 16°C (low light and high temperature). In Antarctic lakes most of the year the autotrophic organisms live at low temperature and irradiance because of the ice-covering, which reduces incident light. Unexpectedly, our results highlighted that the microalga showed the best growth rate at the higher temperature. Moreover this condition did not negatively affect the cell morphology and pigment content. A lower growth rate was instead observed at high light. Furthermore, this irradiance induced the production of high amount of a secondary carotenoid (canthaxanthin) at the end of logarithmic growth and during the stationary phase. The synthesis of this compound is a mechanism activated by *Scenedesmus* sp. to avoid the risk of photoxidative damage to its photosynthetic apparatus. Considering that this metabolite is a commercial high-value compound and that *Scenedesmus* sp. has a relatively fast growth in high light condition, we hypothesize a possible use of this microalga as biotechnological resource.

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Oral Communication Abstract – 8.03

THE SILENCING OF A GST GENE INCREASES THE CONTENT OF HEALTH-PROMOTING DICAFFEOYLQUINIC ACIDS IN TOMATO

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Dicaffeoylquinic acids, pharmaceuticals, VIGS, glutathione S-transferase

There is considerable interest in preventive medicine and food industry in the development of strategies to increase the content of natural antioxidants in edible plants.

Tomato, in addition to lycopene, contains a number of flavonoids and phenolic acids which, synergistically or additively, provide protection against the damage induced by free radicals during oxidative stress, and reduce the risk of certain chronic diseases in human beings.

Among phenolic acids, the mono-caffeoylquinic acids (e.g. chlorogenic acid, CGA) and to an even greater extent the dicaffeoylquinic acids (diCQAs) have been found to possess marked antioxidative properties. However, the development of strategies to increase diCQAs content in plants is hampered by the lack of information on genes involved in their biosynthetic pathways.

Incubation of CGA in crude extracts of tomato fruits led to the formation of two new products, absent in the control reactions (boiled enzyme), which were identified by LC-MS as isomers of diCQAs. We thus hypothesized the presence of a transferase catalysing the synthesis of diCQAs using CGA as acyl donor.

The enzymatic activity increased with advancing fruit ripening, reached the highest value in fully ripe tomato fruits and was accompanied by accumulation of diCQAs.

The enzyme was purified from fully ripe fruits using a combination of ammonium sulphate precipitation and anion exchange chromatography. The final protein fraction resulted in 387 fold enrichment of enzymatic activity, and was subjected to trypsin digestion and mass spectrometric sequencing: the *Tau* Glutathione S-transferase (GST) was selected as a potential candidate gene.

To assess GST functional role, a Virus-induced gene silencing strategy was applied in purple tomato lines which, following expression of the transcription factors *Delia* and *Rosea* from snapdragon, accumulate high level of anthocyanins. These lines make it possible a visual monitoring of VIGS experiments by combining silencing of a candidate gene with that of *Del-Ros* module, which leads to easily recognizable red-coloured sectors within the purple background. As evidenced by LC-MS analyses, red-coloured *Del*/*Ros*/GST-silenced sectors contained 2.1 fold more diCQAs than red coloured *Del*/*Ros* silenced portions. Our results show the involvement of the newly isolated gene in diCQAs metabolism, whose silencing may significantly enhance the content of diCQAs in tomato.

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Oral Communication Abstract – 8.04

THE BEAN *LOW PHYTIC ACID 1* MUTATION IS DUE TO A DEFECTIVE MRP TRANSPORTER, AFFECTING THE REGULATION OF PHYTIC ACID PATHWAY, SEED MYO-INOSITOL CONTENT AND SEED GERMINATION SENSITIVITY TO ABA, BUT DOES NOT IMPACT PERFORMACES UNDER ABIOTIC STRESSES.

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Low phytic acid, common bean, MRP transporter, abscisic acid, abiotic stresses

We had previously identified the *lpa1* (low phytic acid) 280-10 line that carries a mutation conferring a 90% reduction of phytic acid ($InsP_6$) content. In contrast to other *lpa* mutants, *lpa1(*280-10) does not display negative pleiotropic effects. We have now identified the mutated gene and analysed its impact on the phytic acid pathway.

We mapped the *lpa1(*280-10) mutation by bulk analysis on a segregating F_2 population, then, by comparison with the soybean genome we identified and sequenced a candidate gene. $InsP_6$ pathway was analysed by gene expression and metabolites quantification. The mutated *Pvmrp1(280-10)* cosegregates with the *lpa1*(280-10) mutation, and the expression level of several genes of the $InsP_6$ pathway are reduced in the *lpa1*(280-10) mutant as well as inositol and raffinosaccharides content. *PvMrp2*, a very similar paralog of *PvMrp1,* was also mapped and sequenced. The *lpa1* mutation in bean is likely due to a defective *Mrp1* gene (orthologous to the *lpa* genes *AtMRP5* and *ZmMRP4*), while its *Mrp2* paralog is not able to complement the mutant phenotype in the seed. This mutation appears to down-regulate the $InsP_6$ pathway at transcriptional level, altering inositol-related metabolism, and affecting seed germination ABA sensitivity.

The mutant was also tested for drought response and low phosphorous nutrition, showing in both cases no difference in terms of photosynthetic performances or tissue phosphorous content. Finally, a whole transcriptome microarray analysis was carried out for the low phosphorous treatment, for which the data is currently under preliminary evaluation.

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Oral Communication Abstract – 8.05

HOW MUCH THE TRANSGENESIS AFFECTS THE ALLERGENICITY?

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Wheat allergy, wheat kernel proteins, transgenic wheat, proteomics

Food allergy is an adverse reaction to food and represents an important public health problem.

For allergic people low amounts of a food, that is well tolerated by the majority of the population, can cause serious symptoms and even death. The prevalence of food allergy is not well established, but is estimated to be around 6% in young children and 3% in adults. Hypersensivity reaction to wheat flour occurs by inhalation (baker's asthma) and ingestion (food allergy and celiac disease), but may also develop by contact in some cases.

The responsible allergens of wheat allergy are proteins accounting for about 10-15% of the grain dry weight. Wheat proteins are classically divided into two main groups: the salt soluble fraction (albumins/globulins) and the prolamins. This latter fraction is responsible for celiac disease and also for food allergy.The albumins/globulins fraction has also been reported to contain IgE-binding proteins. Since allergies seem increasing, much attention is now being focused on foods from genetically modified (GM) plants because of the postulated risk of allergenicity. In Europe, there is a considerable public resistance to the use of GM technology for crop improvement. This resistance includes the perception that the insertion of transgenes into host plant genomes may result in unpredicted effects on the expression of other genes and effects on plant phenotype (e.g. increases in toxins and allergies). If it is true, transgenic crops could not be considered "substantially equivalent" to non-GM crops.

In order to understand if there is a substantial difference in the accumulation of allergenic proteins in GM wheats (not commercial), sera from children and adults with clinically documented wheat allergy are used for a comparison between a GM-wheat and its wild-type counterpart. The investigation is focused mainly on the soluble protein fraction of wheat. For the comparison, ELISA test and 2D immunoblot are used. Data show that there is no significant difference in the amount of allergenic polypeptides present in the salt-soluble fraction between the GM genotype and its non-transformed counterpart, as well as in the commercial genotype here considered. For comparison, other GM lines have also been analysed.

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Oral Communication Abstract – 8.06

DISSECTING LYCOPENE BIOSYNTHESIS IN TOMATO FRUITS THROUGH VIRUS-INDUCED GENE SILENCING

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Reverse genetic, VIGS, carotenoids pathway, lycopene, S. lycopersicum

Tomato fruits owe their color to the red carotene, lycopene. In order to elucidate the role of the early dedicated genes of the carotenoid pathway, we adopted a Virus-Induced Gene Silencing (VIGS) reverse genetic approach based on agro-infection with Tobacco Rattle Virus (TRV) carrying Rosea1 and Delila silencing fragments (TRV/DR). These serve as a visual marker to dissect silenced from non-silenced sectors (Orzaez et al., 2009). Tomato fruits expressing full-length Rosea1 + Delila, (DR) and consequently accumulating high levels of anthocyanin pigments were infected with TRV/DR, plus a fragment of a carotenoid gene. Seven genes (PSY1, PSY2, PDS, ZDS, ZISO, CrtISO and CrtISO2) were silenced individually in DR fruits and fully ripe fruits were analysed trough HPLC. The results allow to dissect the role of each gene in lycopene biosynthesis and demonstrate that VIGS, associated to a visual marker, is a rapid tool for the functional study of genes controlling tomato fruit quality traits.

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Oral Communication Abstract – 8.07

DEGRADATIVE METABOLISM OF OXALIC ACID AND ASCORBIC ACID IN SPINACH (*SPINACIA OLERACEA* L.).

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Vitamin C, metabolic flux, ascorbic acid degradation, oxalate oxidase, germin like protein

Degradation of ascorbic acid via synthesis and degradation of oxalic acid was studied in spinach seedlings. L-ascorbic-acid (AscA) cleavage at the C_2-C_3 carbon bond is the recognised biosynthetic pathway of oxalic acid (OXA) for oxalate crystal synthesis, but its contribution to the control of the ascorbic acid content of plant tissue and to the control of the content of soluble oxalic acid in plant tissues is poorly known. OXA in plants can be degraded, by the enzymatic activity of oxalate oxidase (OXO, EC 1.2.3.4), in carbon dioxide and hydrogen peroxide nevertheless, the knowledge about the regulation of activity of this enzyme are largely lacking. AscA is present in all plant species and oxalate is widely distributed in the plant kingdom both as a soluble acid or insoluble salt, mainly calcium oxalate. Both, AscA and OXA, play important physiological roles in plants, are metabolically linked and possess opposite nutritional value for human health. The metabolic link between AscA and OXA and the biochemistry of OXA degradation in plant food are important in terms of plant physiology and nutritional quality of plant derived food. After feeding with 125 mM of AscA or OXA for 4, 10, 24 h, and after 4, 8, 24 h of further water feeding (washing), changes of ascorbic and oxalic acid concentration, of the activity of oxalate oxidase, of the gene expression of a putative H_2O_2 –producing oxalate oxidase germin like protein, were measured in spinach seedlings. Ascorbate or oxalate treatment produced a time-dependent accumulation of AscA and soluble OXA in hypocotyls and, at higher levels, in cotyledons of spinach seedlings. During washing, AscA and OXA contents decreased at a very fast rate. After feeding 99% $^{13}C_2$ AscA, the $^{13}C/^{12}C$ ratio in the CO_2 released by spinach tissues increased. An insoluble form of OXO was preliminarily found in different spinach tissues, including hypocotyls and cotyledons. The insoluble OXO activity increased several fold following metabolite feeding in both hypocotyls and cotyledons and remained high during washing. A soluble form of OXO appeared in the tissues, after AscA and OXA feeding. AscA and OXA feeding induced the gene expression of a putative OXO germin like protein. Our results suggest that a) AscA can be degraded in spinach tissues via OXA synthesis and that OXA can then be degraded to H_2O_2 and CO_2 via OXO activity, b) the mentioned degradative pathway can be induced by AscA and OXA feeding in a feed-forward mode, via the increase of OXO activity d) the increase of OXO activity is linked to the increase of gene expression of a putative germin like protein. We demonstrate that the AscA and soluble OXA pools are metabolically linked in spinach tissues, and that AscA can be degraded at such a fast rate that the amount normally present in spinach tissue can be cleaved in a few hours.

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Poster Communication Abstract – 8.08

EVOLUTION OF THE GENETIC STRUCTURE IN *TRITICUM DURUM* DESF. GERMPLASM FROM SOUTHERN ITALY

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Triticum durum, genetic resources, morphological traits, SSR, conservation measure

Among the Mediterranean countries, Italy has the longest tradition in durum wheat (Triticum durum Desf.) breeding and its germplasm can be considered as one of the richest and most valuable. There are indications that in the past decades the genetic bases of this crop may have been eroded coupled with the diffusion of a relatively small number of outstanding genotypes with proven adaptability and yield potential. Over time large germplasm collections have been established and now "old type" germplasm can be recovered from genebanks. In southern Italy durum wheat germplasm has been collected starting from the 1947 up to year 2000 and the changes of the genetic structure of this precious wheat germplasm that occurred over time have been studied on the basis of morphological traits and molecular markers, particularly SSR.

A sample of 107 durum wheat accessions collected since 1947 in Southern Italy was analyzed at the University of Basilicata by 22 quanti-qualitative morphological traits and 30 microsatellite loci. The accessions were grouped into two groups on the basis of their collection date.

A great variability was observed for morphological traits, particularly for quantitative ones. These characters showed a significative differentiation among the two groups.

Molecular markers revealed high polymorphism and identified 115 alleles, with an average of 3,83 alleles per locus. Nine private alleles were found in the first group, while in the second one they were five; it was also possible to identify accessions with rare alleles, mostly of the first group. Molecular analysis revealed a decrease of the genetic diversity over time.

The use of morphological and molecular markers revealed of great utility in assessing temporal trends in the diversity of Southern Italy wheat germplasm. The evidence of both genetic diversity and genetic erosion of durum wheat genepool further strengthens the strategic relevance of undertaking appropriate genetic conservation measure either at local or global scale.

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Poster Communication Abstract – 8.09

SCREENING OF FAVOURABLE ALLELES FOR β-CAROTENE CONTENT IN MAIZE INBRED LINES

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Zea mays L., inbred lines, β-carotene, hydroxylase3, PCR

Carotenoids are natural compounds that play an important role for human nutrition and health; among them β-carotene is quite relevant, being a precursor of vitamin A. The nutritional value of staple crops like maize could be improved by provitamin A biofortification. A simple PCR assay was developed, that can be used to identify the alleles of the gene *hydroxilase3* associated with a enhanced or reduced provitamin A content (Vallabhaneni *et al*., 2009). The presence of alleles B or C of this gene, which is a hydroxylation gene codifying for a key enzyme in carotenoids pathway, could be considered a marker of a high β-carotene content in maize kernels. Eighty-six Italian inbred lines, selected as starting material for a breeding program focused at improving maize nutritional quality, were analysed by this method. Molecular analysis brought up to the identification of 11 lines carrying alleles B or C. Introgression of these alleles was tested in ten hybrids derived from crosses among these same lines. Total carotenoids were extracted from a set of inbreds and quantified by spectrophotometric analysis at 450 nm. Preliminary data revealed a range of variation from 14.67 μg g^{-1} dm to 40.67 μg g^{-1} dm. The evaluation of the ratio of provitamin A (α- and β-carotene) to non-provitamin A compounds (cryptoxanthin, lutein and zeaxanthin) is currently being carried out by thin-layer chromatography (TLC).

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Poster Communication Abstract – 8.10

COMPARATIVE PROTEOMIC ANALYSIS OF METABOLIC AND STARCH GRANULE-ASSOCIATED PROTEINS IN WHEAT KERNEL OF A HIGH AMYLOSE TRANSGENIC LINE AND ITS CORRESPONDING UNTRANSFORMED CULTIVAR

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Starch, high-amylose, transgenic wheat, proteomic

Wheat is widely cultivated in the world and represents one of the most important cereal for the human diet. The components that mainly influence semolina and flour quality are the proteins and the starch. Starch is of remarkable interest because it can be used for various applications in food and non food industry. It is composed by amylose and amylopectine, whose relative amounts affect its chemical-physical properties and final uses, In the last years various studies have been performed to clarify the mechanisms that regulate starch biosynthesis, in order to produce starches with specific properties, such as a higher amylose content, that has beneficial effects on human health.

In our laboratory, high amylose transgenic lines of durum wheat have been produced through the silencing of the Starch Branching Enzyme IIa (*SBEIIa)* genes. In this work, a 2D electrophoretic comparison between the kernel proteome of a transgenic line of the durum wheat cultivar Svevo with silenced *SBEIIa* genes and the corresponding untransformed line have been performed. In particular, two developing stages have been considered: the dough (15 dpa) and ripening (physiological maturity) stages.

As for the mature stage, the differentially expressed proteins have been identified by the software SameSpots Progenesis (Nonlinear Dynamics, UK) and characterized by MS/MS. The analysis of proteins associated to starch grains have revealed three polypeptides (a phosphorylase, a b-amylase and a ramification enzyme class I (SBEI)), that show a higher volume in the transgenic line with respect to cv. Svevo. This could be the resultof a compensating effect of the absence of the isoform SBEIIa. Moreover, four protein spots are present exclusively in the transgenic line and absent in Svevo. MS/MS analysis identified these polypeptides as Granule Bound Starch Synthase (GBSS) fragments.

Based on the analyses of proteins of the soluble fraction of the mature wheat kernel, corresponding to the so-called metabolic proteins, seven spots whose volumes are lower in the transgenic line respect to the control, have been also characterized by MS/MS. Among these proteins, some are involved in carbohydrate metabolism (glyceraldeyde 3-phosphate dehydrogenase, fructose bi-phosphate aldolase and b-amylase); others have a defense role against biotic and abiotic stresses (a-purotionine, a-amylase inhibitor, 3C globulin, HSP70), and these latter are also known for triggering adverse reaction in sensitive individuals.

The analyses relative to the dough stage of wheat kernels are under investigation.

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Poster Communication Abstract – 8.11

POLYMORPHISM OF STARCH GRANULE PROTEIN 1 (SGP-1) IN POLYPLOID AND DIPLOID WHEAT SPECIES

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Starch, sgp-1, wheat, polymorphism

Wheat reserve starch is produced by the concerted action of 4 different starch synthases, branching and debranching enzymes. Starch synthase IIa is a granule bound enzyme (known as Starch Granule Protein 1, Sgp-1) that plays an important role in amylopectin biosynthesis. In fact the knockout of Sgp-1 proteins is correlated with a high amylose content in cereals, such as wheat, rice and barley.

In this paper SDS-PAGE analysis permitted to identify novel polymorphisms for Sgp-1 proteins in diploid and polyploidy accessions. In particular we focused on A-, B-, and D-genome diploid ancestors (*T. urartu*, *Ae. speltoides* and *T. tauschii*) and on tetraploid and hexaploid cultivated species (AABB *T. turgidum*, AAGG *T. timopheevii* and AABBDD *T. aestivum*). A different electrophoretic mobility has been found between Sgp-1 of wild (*T. urartu* and *T. monococcum ssp boeoticum*) and cultivated (*T. monococcum ssp monococcum*) diploid wheats with A genome. Sgp-A1 proteins of *T. urartu* accessions have a SDS-PAGE mobility similar to those of tetraploid and hexaploid species, but they have an higher molecular weight than that of cultivated diploid accessions (*T. monococcum ssp monococcum*). In order to clarify this different mobility, the entire codon region of the two genes has been isolated and sequenced. Differences between deduced amino acidic sequences have been found. Moreover two accessions of *Ae. speltoides* resulted to have Sgp-S1 proteins with different molecular weight on SDS-PAGE gel, similar to Sgp-B1 of polyploid wheats and Sgp-G1 of *T. timopheevii*, respectively.

No polymorphism has been identified in D-genome ancestor accessions (*T. tauschii*) in comparison to Sgp-D1 of *T. aestivum*.

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Poster Communication Abstract – 8.12

ACCUMULATION OF CM PROTEINS IN OLD AND NEW DURUM WHEAT CULTIVARS UNDER DIFFERENT NITROGEN FERTILIZATION

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Allergies, durum wheat, mass spectrometry, CM proteins nitrogen fertilization

Wheat represents the cereal crop mainly used by the humankind. However, it belongs to the group of six foods, identified by the Codex Alimentarius, as eliciting IgE-mediated allergic responses such as skin pathologies, gastrointestinal and respiratory disorders. The allergic diseases (bakers' asthma, different kind of dermatitis and food allergy, including exercise-induced anaphylaxis) are induced by wheat kernel proteins. These latter are also responsible of technological and nutritional properties of wheat flours, as well as those of the derived foods.

Wheat kernel proteins are grouped into two major classes: the gluten proteins and the soluble proteins (albumins and globulins). Among these latter, there are also the so-called chloroform-methanol soluble (CM), greatly involved in baker's asthma.

There is a matter of debate regarding the supposed increase in wheat allergies. If this is real or it is the result of the improved diagnostic systems, is unclear. It is certain that plant breeding has caused a dramatic increase in protein content that, of course, involves a higher expression of allergenic proteins. This is particularly evident in the modern durum wheat varieties, that can reach protein contents of about 18% as opposed to the lower values observed in the old cultivars.

Protein content is strongly influenced by available nitrogen that is usually supplied by means of fertilization.

The aim of the present work is to characterize CM proteins of four durum wheat genotypes, two of which correspond to modern Italian varieties (Svevo and Claudio) and two to old Italian varieties (Senatore Cappelli and Urria). These genotypes have been grown in Florence (Italy) with three levels of nitrogen fertilization.

A proteomic comparison (4 genotypes X 3 biological replicas X 3 technical replicas X 2 nitrogen levels=108 2D gels in total) is being performed on the CM fractions of the four genotypes.

Data about such comparison will be presented.

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Poster Communication Abstract – 8.13

LIPOXYGENASE ACTIVITY IN *TRITICUM DURUM*: DIFFERENTIAL PROPERTIES OF ENZYME FORMS

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Durum wheat, lipoxygenases, carotenoids, lutein, hydroperoxide stereochemistry

Durum wheat (*Triticum turgidum* L. ssp. durum) is the main cereal crop in the Mediterranean area. It is used to produce pasta, for which a proper yellow colour is an important quality parameter that increases buyer confidence and product value. The colour is given by the presence of carotenoid pigments in semolina, mainly lutein. However, a significant loss of these pigments may occur during pasta processing as a consequence of the activity of lipoxygenases (LOX, linoleate:oxygen oxido-reductase, EC 1.13.11.12). The hydroperoxides produced generate in turn volatile compounds, which give undesirable flavour to foodstuff. The presence of lutein is not only an aesthetic parameter of pasta products, but has also a nutritional value. Carotenoids in the diet reduce the risk of ocular diseases, including the age-related macular degeneration, and have been recently related also to an increased protection from developing cardiovascular diseases and several types of cancer. Therefore the enhancement of lutein content in durum wheat may produce beneficial effects on human health, particularly for those populations for which the daily intake of carotenoids is well below the recommended dose.

Lipoxygenases are non-heme iron-containing dioxygenases present also in microorganisms and animals. Their substrate in plants is represented mainly by linoleic, linolenic and arachidonic acids. Several enzyme forms showing distinct biochemical properties (pH optimum, substrate range and affinity, product stereospecificity) have been reported in several species. Two isoenzymes have been purified and characterized in barley (Holtman *et al*., Plant Physiol. 111, 569-576, 1996). LOX1 produces mainly 9-hydroperoxide-octadecadienoic acid, whereas LOX2 converts linoleic acid into 13-hydroperoxide-octadecadienoic acid. LOX-1 is present in both quiescent and germinating seeds and is responsible for most activity in mature grains, whereas LOX-2 is found only at early stages of grain development and after germination. Specific features suggest specialized functions, yet the role of each isoform has not been fully elucidated.

Within the frame of a research project aimed at improving our knowledge on the biochemical bases of carotenoid content in durum wheat and pasta products, LOX activity was characterized in *T. turgidum* ssp. durum cv Ofanto. Recent data at the molecular level showed the presence in this species of no less than five genes with different allelic variants, some of which were associated with a strong reduction in LOX activity in semolina (Verlotta *et al*., BMC Plant Biology 2010, 10:263). However, no direct evidence of a differential catalytic rate has been obtained to date. Two isoforms were resolved in extracts from suspension cultured cells by ion-exchange liquid chromatography. Biochemical characterization carried out with three different assay methods showed diverse functional properties, coupled with a distinct pattern of expression during the cell culture growth

cycle. Experiments are in progress in order to purify isoforms to electrophoretic homogeneity, and to investigate their carotenoid bleaching activity.

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Poster Communication Abstract – 8.14

A STUDY OF BIODIVERSITY OF PHENOLIC CONTENT IN THE WHEAT CARYOPSIS

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Pigmented wheat, phenolic biodiversity, antioxidant compounds

Pigmented cereals, including pigmented wheat, are interesting for the production of functional foods, since the caryopsis colour is associated with total phenolic content, which, in turn, is highly correlated with the total antioxidant capacity. Wheat is the most important cereal in our diet, therefore the use of this antioxidant-rich cereal for the production of bakery products would import them an high nutritional quality. Moreover, pigments, although in very low concentration, can significantly affect the quality of bread and pasta.

We have analyzed bread wheat and durum wheat genotypes from two harvests; these different pigmentation of the pericarp or aleurone layer: the durum wheat germplasm included genotypes with red or purple pericarp and blue aleurone, while the bread wheat germplasm tested icluded purple and blue aleurone genotypes. The samples were analysed for their polyphenol content and their technological behaviour. The results evidenced that pigmented wheat are often characterized by a polyphenol content higher than that of the genotypes normally cultivated.

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Poster Communication Abstract – 8.15

DEVELOPMENT OF REAL-TIME PCR ASSAYS FOR THE DETECTION OF ALLERGENIC SPECIES IN FOOD

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Food allergens, Multiplex Real-Time PCR, SYBR Green, food labelling

Food allergies are a major health concern in industrialized countries and may affect up to 3% of the adult population and 6-8% of the children in Europe. A large number of anaphylactic reactions to food are treated in emergency department each year, and it is estimated that food allergy causes several deaths annually. The level of exposure necessary to provoke a reaction varies from food to food and from person to person. Most often, reactions are elicited after exposure to 1-100ppm of an allergen, but sometimes, only minute amounts are required. Thus, the European Commission proposed the European Food Labelling Directive 2000/13/EC, 2003/89/EC and 2006/142/EC. The proposal contains a list of liable ingredient to cause allergies. The EU allergen list is intended to be dynamic, and more allergens may be included over time. Detection of hidden allergens may be difficult by the consumer, because of products mislabelling or unintentional cross-contamination during food production. Any molecule that is specific for the allergenic ingredient can serve as a marker of its presence in food: mostly protein and DNA are targeted for the purpose. DNA analysis, as compared to protein, is more specific, reproducible, sensitive, rapid and inexpensive. DNA is also highly stable during food processing; DNA-based tests have proven to be very useful to authenticate the species used in foodstuff production.

This study describes the comparison of several DNA-extraction methods utilised on several food matrices like nuts, fruits and vegetables and on different food products such as biscuits, yogurt and baby foods. PCR, Real-Time PCR and Multiplex PCR with SYBR®GreenER™ assays have been designed to specifically detect different allergenic plant species, and they have been tested on several commercial food products.

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Poster Communication Abstract – 8.16

MOLECULAR CLONING AND CHARACTERIZATION OF ALLERGENIC PROTEINS FROM MAIZE

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Food allergy, Zea mais, recombinant allergens, component resolved diagnosis, immunotherapy

Food allergies are a problem of considerable social impact and increasing diffusion. As part of a larger project (PRIN 2008) aimed at the identification and characterization of allergenic proteins present in low concentrations in foods of plant and animal origin, the research presented in this communication has the specific objective to identify genes coding for allergenic proteins from maize, and the production of recombinant allergens. This type of study fits into the research lines of modern allergology. Indeed, the possibility of obtaining recombinant allergenic proteins opens the possibility of future applications both in diagnosis and therapy [1]. The recombinant allergens can be used in diagnostic tests, instead of natural allergen extracts, eliminating the problem of variability and allowing the accurate determination of the molecular profiles of patients allergic sensitization (component resolved diagnosis). In addition, the availability of recombinant allergens makes it possible strategies of immunotherapy based on the development of hypoallergenic allergens, obtained by applying molecular biology tools (expression of molecular sub-regions, mutagenesis, synthesis of variants of protein folding, gene-shuffling, etc.).

Despite the wide consumption, maize has only recently been described as a cause of allergy (see [2] and references cited within). The first characterized allergen was a "lipid transfer protein" (LTP) of 9 kDa. More recently other proteins have been described as maize allergens: vicilin, globulin-2, gamma-zein, endochitinase, thoredoxin and trypsin inhibitor [2]. The case of maize LTP allergen is particularly interesting because the protein is known to bind IgE even after heating to 100 °C.

We have focused our initial efforts on the proteins LTP (EBI, Q2XX13). The coding sequence has been cloned in different vectors for expression in both *Escherichia coli* and *Pichia pastoris*.

Purified protein will be tested to confirm its allergenic activity, a necessary prerequisite for inclusion in the list of allergens of the International Union of Immunological Societies. Mutagenesis studies will then be initiated and assessment of allergenicity of new forms of allergen established. The analysis of LTP multigene families in different species of maize (*Zea diploperennis*, *Z. luxurians*, *Z. mays*, *Z. nicaraguensis*, *Z. perennis*) by a Genome Walking technique developed in our laboratories [3] is also in progress, so to assess the distribution and prevalence of the different isoforms of the protein.

The same experimental approaches will be used for other maize allergenes.

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Poster Communication Abstract – 8.17

LOW PHYTIC ACID 1 MUTATION IN MAIZE, NOT ONLY A PHOSPHOROUS ISSUE

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Maize, phytic acid, starch, ions, proteins

Phytic acid, myo-inositol 1,2,3,4,5,6-hexakisphosphate (IP6), is the major storage form of phosphorous in plants and it is mainly accumulated in seeds (up to 4-5% of dry weight) and pollen. In maize kernel the 80% of phytic acid is localized in the scutellum while the remaining 20% is in the aleuronic layer. Phytic acid is deposited as mixed salts of mineral cations in protein storage vacuoles and during germination it is idrolized by phytases. Phytic acid and the cations that it is able to bind are poorly bio-available for monogastric animals due to their lack of phytase activity, for this reason phytic acid P is mainly excreted in wastes, furthermore the nutritional value of the seeds decreases due to the low bio disponibility of micronutrients which are important feed/food components. The undigested phosphorous contained in excreted phytic acid can also contribute to water pollution.

One strategy to solve this problem is the isolation of *low phytic acid* (*lpa*) mutants able to accumulate low level of phytic P and high level of free phosphate in the seeds while the total content of seed P is not modified. Among cultivated plants maize is one of most important, it is used for many purposes, in several countries as staple food, as feed for animals and in industrial activity. Three *low phytic acid* maize mutants have been isolated: *lpa1*, *lpa2* and *lpa3*; *lpa*1 exhibited the most relevant reduction of phytic acid in the seed.

The *lpa* mutations influence not only the phosphorous accumulation in the seed but also the plant development and its productivity due to negative pleiotropic effects. This can reflect the involvement of inositol phosphates such as phytic acid in fundamental biological process.

Here we present genetic, physiological and hystological data regarding *lpa1-7*, a *low phytic acid 1* mutant allele, obtained by chemical mutagenesis. We further investigate the effect of the *lpa1* class of mutations on several aspects of grain quality, such as storage proteins, ions accumulation and nutritional value. We also highlight differences in starch content, structure and functional properties.

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Poster Communication Abstract – 8.18

STUDY OF MAIZE GENOTYPES RICH IN ANTHOCYANINS FOR HUMAN AND ANIMAL NUTRITION

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Maize, anthocyanin, functional food, epigenetics

Flavonoids, a group of secondary metabolites belonging to the class of phenylpropanoids, play essential functions in plants. Besides, epidemiological studies suggested that regular consumption of flavonoid-rich foods or beverages is associated with a decreased risk of cardiovascular mortality. Among the flavonoids, it seems that anthocyanins might function as potent in vivo antioxidants: their long-term dietary potential health benefits can be proved by the use of plants that accumulate specific anthocyanins.

Maize could be an example of these functional foods: purple and blue corn are pigmented varieties rich in anthocyanins originally cultivated in South America. In maize, anthocyanins are synthesized by a complex pathway made up of more than 20 genes and regulated by two classes of transcription factors, *R1/B1* bHLH genes and *C1/Pl1/P1* MYB gene families. The presence of dominant alleles of these regulatory genes is necessary to accumulate the pigments.

The introgression of these alleles in european inbred lines and populations has been made for years in our experimental field. In this project NILs (Nearly Isogenic Lines), derived from six cicles of self-pollination after a cross between an inbred line and a line carrying the regulatory genes for anthocyanin accumulation in the pericarp (*B* and *Pl*), were studied. An unexpected variability in the anthocyanin content was discovered among the flours obtained from seeds of each ear of the NIL; this variability was due neither to differences in the pericarp thickness nor in the mean seed weights.

An epigenetic phenomenon was hypotesized to explain this variability. It is known in fact that the regulatory genes of the anthocyanin biosynthetic pathway are susceptible to silencing processes, thus the variation could be due to a partial switch off of the anthocyanin pathway genes. Preliminary results of regulatory and structural genes expression analysis seemed to support this hypothesis, however further experiments, such as azacitidine treatments and methylation –sensitive enzymes tests, are planned.

These results could allow a better understanding of the regulatory mechanisms of the anthocyanin pathway, with the aim to improve the genetic selection to obtain corn lines with the highest amount of these pigments.

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Poster Communication Abstract – 8.19

NUTRITIONAL QUALITY IMPROVEMENT IN COMMON BEANS BY GENETIC REDUCTION OF PHYTIC ACID AND OTHER ANTINUTRITIONAL FACTORS

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Phytic acid, antinutritional factors, lpa280-10 mutant

When common bean grains are consumed, phytic acid, raffinosaccharides, polyphenols and tannins exert major antinutritional effects such as reduced phosphate, iron and zinc absorption, low protein digestibility, and flatulence. Moreover, if cooking is not properly carried out, lectins and protease inhibitors may exert toxic intestinal effects.

In order to improve the nutritional characteristics of bean grain used for human consumption and potentially for feeding monogastric animals, we developed several innovative bean lines. First, we developed several lectin-free (***lf***) lines producing coloured seeds, then we combined the ***lf*** and the ***wsc*** (white seed coat, correlated with very low amounts of tannins and polyphenols) traits in the same genetic background. Finally, we introduced the low phytic acid (***lpa280-10***) trait in the ***lf*** and ***lf*** + ***wsc*** backgrounds. Since it is well known that *lpa* mutations may cause negative physiological effects in bean seeds, in particular lower seedling emergence and thus lower grain yield, we submitted the new bean lines to a field performance test carried out in two Italian locations, as well as to biochemical analyses and bioassays aimed to evaluate their nutritional and technological characteristics.

Obtained results were as follows :

1. The introgression of the *lpa* mutation caused large phytate reductions (80-90%) without affecting yield or introducing negative agronomical effects.

2. Compared to a suitable control line, bio-availability of bean grains iron (assayed by the in vitro digestion/Caco-2 cells bioassay measuring the amount of accumulated ferritin) was enhanced 2.5 folds in a line with a low content of tannins and polyphenols and exhibited a further 2.5 folds increase in a line with the same background additionally endowed with the low phytate trait. Moreover, unexpectedly, in the new beans we found a remakable increase of protein content.

3. Statistical analyses of the obtained biochemical data revealed a number of highly significant correlations between the 11 investigated parameters, some of which were expected (such as the positive interactions Fe/Zn, tannins/polyphenols), while others (for

example the negative interactions protein/Pi, protein/polyphenols) need further investigation to be interpreted.

Further testing and development of these breeding lines are underway to confirm the *in vitro* observations in an animal model.

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Poster Communication Abstract – 8.20

APPLICABILITY OF SSR MARKERS TO THE TRACEABILITY OF MONOVARIETAL OLIVE OILS

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Olive oil, food genomics, SSR markers, shortened SSR, DNA extraction

To protect the features and authenticity of food products, the European Commission enforces two certification labels: Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI). EEC Regulation No. 510/2006 imposes criteria for labelling, production and commercialisation of olive oil.

Since plant genotype is a major determinant in establishing the PDO and PGI labels, methods to ascertain the varieties present in a batch of olive oil are essential in validating product conformity.

The traceability of olive oil can be assessed through simple sequence repeat (SSR) co-dominant markers targeted to specific regions of DNA from olive cultivars.

Twenty-one monovarietal olive oils were analysed with nine nuclear and two shortened SSRs. For each marker the correspondence of allelic profile with the reference cultivar, the reproducibility of profiles in different DNA extractions and the polymorphism information content were determined.

The results showed that using a panel of SSR markers such as those described allows one to make a reliable attribution of an olive oil to a specific cultivar.

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Poster Communication Abstract – 8.21

INVESTIGATION ON A (+)-GERMACRENE A SYNTHASE INVOLVED IN SESQUITERPENE LACTONES BIOSYNTHESIS IN GLOBE ARTICHOKE

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Cynara cardunculus, (+)-germacrene A terpene synthase, sesquiterpene lactones

Globe artichoke (*Cynara cardunculus* var. *scolymus* L., *Asteraceae*) is a perennial crop traditionally consumed as a vegetable in the Mediterranean countries and rich in nutraceutically and pharmaceutically active compounds. Its bitter taste is caused by its high content of sesquiterpene lactones (STLs), a class of compounds which have also been shown to be medicinally active as antihyperlipidemic, anticancer, antispasmodic and antimicrobial agents.

The biosynthetic pathways responsible for STL production remain largely unknown, but in other *Asteraceae* species the initial enzymatic step is known to consist in the cyclization of farnesyl diphosphate (FPP) by the terpene synthase (+)-germacrene A synthase (GAS).

Here, we have mined a set of ~19,000 globe artichoke unigenes to identify a putative globe artichoke *GAS* orthologue. An alignment of its sequence with those of other plant sesquiterpene synthase genes highlighted the conserved peptide motifs DDxxD and RxR, characteristic of the enzymatic family members. When heterologously expressed in *E. coli,* the putative globe artichoke GAS was able to convert FPP into ß-elemene, a rearranged version of (+)-germacrene A.

The level of expression of the isolated GAS gene was assayed by quantitative RT-PCR in globe artichoke tissues. Among various tissues of the plant assayed (2-6 and 20 weeks-old leaves, head bracts and receptacles and 'in vitro' calluses), the level of globe artichoke *GAS* expression was highest in mature (six week old) leaves. Moreover, a sequence polymorphism within a mapping population parent allowed the *GAS* locus to be placed on the genetic map we previously developed.

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Poster Communication Abstract – 8.22

CHARACTERIZATION OF LIPASE ACTIVITY IN GREEN COFFEE BEANS DURING STORAGE AND GERMINATION

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Lipase, green coffee, germination, storage, lipid

Coffee seeds possess an intermediate storage pattern, showing a non-quiescent behaviour characterized by various metabolic reactions occurring during storage. In particular, lipase is the main enzyme involved in the mobilization of triacylglycerols, providing energy and a source of carbon skeleton during early stages of germination. During storage, the triacylglycerols might be involved in the generation of undesirable compounds (known as "off-flavours"), lowering both the viability of coffee seeds and the cup quality. In this work, the soluble protein fraction from coffee seeds and plantlets was extracted by acetone and then was utilised to assay lipase activity. Green coffee beans, harvested in Colombia, were stored at room temperature for 2-3 months, in order to verify the influence of prolonged storage on lipase activity. For germination experiments, the seeds were imbibed for 7 days at 30 °C and transferred in perlite at 28 °C and 90% R.H. for further 3 weeks. Lipase activity was detected by a colorimetric method based on specific degradation of a chromogenic substrate, at pH 8.2. Green coffee seeds exhibited an appreciable lipase activity that was slightly increased during storage. Such an activity was inhibited by tetrahydrolipstatin (THL) in a concentration-dependent manner, while it was slightly stimulated by both EGTA and EDTA. During the germination, after 10, 14, 17 and 21 days, lipase activity showed an initial increase that was followed by a gradual decrease. The effect of the presence or absence of the parchment (seed coat), during the first stages of germination, has also been investigated.

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Poster Communication Abstract – 8.23

LAMB'S LETTUCE (*VALERIANELLA OLITORIA* [L.] POLLICH) STORAGE AT LOW TEMPERATURE IS IMPROVED BY PARTITIONED LIGHT

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Bioactive compounds, cold storage, green tissues, lamb's lettuce, light treatment

Light treatments during storage at low temperature might induce both positive and negative effects on shelf life and quality of produces. For this reason, in this work we examined the effect of low light irradiance on lamb's lettuce during storage at a low temperature. Partitioned light treatments (4 doses per 1h per day; 8 doses per 1h per day; 16 doses per 0.5h per day) showed positive effects, while continuous light treatments (8h per day) were deleterious. The content of photosynthetic pigments, energy-linked metabolites and antioxidants was evaluated at the beginning and after 6 days of storage in comparison with samples stored at 4°C in the dark. The content of such bioactive compounds was increased or at least similar in samples stored under partitioned light and at a higher temperature (6°C) when compared to those stored in the dark. We suggest that continuous light treatments could promote photosynthesis but also cause photo-damage during cold storage of lamb's lettuce. However, the photosynthesis under partitioned low light is only partially activated and so the photo-damage is limited, although the metabolism of green tissues would be still able to provide carbon moieties for the synthesis of bioactive molecules, thus delaying senescence. With respect to the samples stored in the dark at a lower temperature (4°C), partitioned low light treatments at 6°C could contribute to ameliorate the quality of lamb's lettuce and, at the same time, allow an energy saving.

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Poster Communication Abstract – 8.24

HPPR GENE IDENTIFICATION IN *SALVIA OFFICINALIS* CELL CULTURES FOR THE PRODUCTION OF ROSMARINIC ACID

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Hydroxyphenylpyruvate reductase (HPPR) gene, Salvia officinalis, Rosmarinic acid, cell cultures, nutraceutical

Several bioactive substances have been recently taken into consideration for their applications as food preservative, nutraceuticals and for pharmaceutical purposes due to functional properties as antioxidant and antimicrobial compounds. Plant species belonging to Lamiaceae and Boraginaceae are very rich in rosmarinic acid (RA), an ester of caffeic acid with 3,4-dihydroxyphenyl-lactic acid. The presence of RA in medicinal plants, herbs and spices has beneficial and health promoting effects, for its interesting biological activities, e.g. antiviral, antibacterial, antiinflammatory and antioxidant agent. RA is supposed to act in plant as a defence compound. The biosynthesis of RA has been recently defined: HPPR (hydroxyphenylpyruvate reductase) is considered the first specific enzyme and represents the key-enzyme responsible for this metabolic pathway.

In this work *Salvia officinalis* L. cell cultures were studied in order to 1) analyse the production of antioxidants and RA, 2) isolate a cDNA and identify the hydroxyphenylpyruvate reductase (HPPR) gene, 3) evaluate the use of the HPPR gene as molecular marker for the controlled production of the metabolite of interest.

A specific *S. officinalis* cell line was selected for the high antioxidant capacity of its hydroalcoolic extract, which was characterized by a very high content of RA. The antioxidant total capacity (DPPH test) and the total RA content were evaluated during the cell growth curve related to the developmental phase.

cDNA obtained from *Salvia officinalis* L. cell cultures was then analysed by PCR, using pairs of specific and degenerate primers built on the basis of information in GenBank. The results of the sequencing of PCR products have been successful in aligning with the software BLAST.

cDNA partial sequence of the (HPPR) gene was then isolated from *Salvia officinalis* cell cultures (GenBank: EU924744.1). HPPR gene expression is correlated to the production of RA in cell cultures.

So far the identification of the omologous cDNA sequence of the HPPR enzyme in suspension cultures of *Salvia officinalis* can increase the knowledge of RA biosynthesis in this plant and act as a marker of the antioxidant activity.

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Poster Communication Abstract – 8.25

GLUTATHIONE OXIDO-REDUCTIVE STATE INTO *BRASSICA RAPA* L. CV. *SYLVESTRIS* DURING POSTHARVEST STORAGE

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Postharvest storage, glutathione, thiols, Brassica rapa L. cv. sylvestris

Leafy vegetables at harvest suffer a strong stress condition due to the block of water, nutrient and hormone flow. The cutting, in fact, leads to the production of ROS (reactive oxygen species) that activate programmed senescence and/or repair processes both locally and systemically. The control of the oxidative stress involves enzymes and metabolites with antioxidant activity such as superoxide dismutase and catalase, ascorbate, glutathione, tocopherols, carotenoids and phenol compounds.. A key role is played by glutathione (GSH) a sulphur compound that can react with ROS as antioxidant and as intermediate of the the ascorbate cycle. It regenerates ascorbate through the dehydro-ascorbate reductase. The oxidised glutathione (GSSG) is then reduced to glutathione (GSH) by the NADPH dependent glutathione reductase. Glutathione, on the other hand has a key role in the floem distribution of sulphur compounds and synthesis of sulphur secondary products. In this view the evaluation of glutathione and its redox state is essential to understand the plant organs physiological state.

In this study glutathione and its redox state (GSH/GSSG) as well as for other sulphur metabolites were determined during postharvest storage of the the edible part of Brassica rapa L. cv. Silvestris (friariello napoletano). The top parts of the plants were collected at crop maturity. placed in plastic trays and stored at different temperature (4 °C, 9 °C and 20 °C). Samplings, in triplicate, were made at harvest and during storage up to 20 d. In orgder to evaluate the effect of storage on different organs, the samples were separated in floret, leaf and stem, quickly frozen in liquid nitrogen and stored at -80 °C. The sulphur compounds were determined on acid extracts by derivatization with monobromobimane (MBB). Being the analysis of reduced sulphur compounds in tissue extracts very difficult because they are rapidly oxidized by molecular oxygen, leading to an underestimation of the reduced form and, therefore, of their redox state within the cell, the derivatization reaction with (MBB) was done by an automatic method assisted by the HPLC autosampler. The online separation and quantification of the fluorescent derivatives were done by HPLC. The results suggested that in plant tissues stored at 4 ° C glutathione was highly reduced at harvest. During postharvest it firstly decreased when the ascorbate level in the tissues was high and subsequently increased, evidencing that it could be highly involved in the oxidative stress control in the first period of storage. Such pattern occurred in leaf blade and stem, but not in the floret tissue that showed unchanged levels of glutathione up to 8 d of storage at 4°C.

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Poster Communication Abstract – 8.26

CHANGES ASCORBATE PEROXIDASE GENE EXPRESSION POST-HARVEST IN *BRASSICA RAPA* L.

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Ascorbate peroxidase, gene expression, semi-quantitative RT-PCR

Broccoli is a very good source of dietary fiber, vitamins A, C, K, and B6, folate, and manganese. Additionally, many studies indicate that a regular intake of broccoli has a strong correlation with cancer prevention and inhibition [1]. Broccoli is harvested at an immature stage before growth has ceased. Broccoli is known as an ascorbate rich vegetable, although rapid degradation of ascorbate has been seen to occur in florets at ambient temperatures after harvest decreasing the nutraceutical proprieties. It is well known that ascorbate plays essential roles as an antioxidant and a cofactor of many dioxygenases which determine many important steps of cell metabolism in plants and animals [2]. Harvesting can result in considerable damage due to the sudden disruption in water, energy, nutrient, and hormone supplies and can cause wounding stress in plants. In order to understand the regulation of the ascorbate level, it is important to determine the alteration of ascorbate related enzyme activities or gene expressions under harvest. A well recognized enzyme consuming ascorbate is ascorbate peroxidase (APX), which catalyzes the reduction of hydrogen peroxide to water with simultaneous oxidation of ascorbate with a high specificity. APX isoenzymes are distributed in at least four distinct cellular compartments: stromal APX (sAPX) and thylakoid membrane bound APX (tAPX) in chloroplasts, microbody (including glyoxysome and peroxisome) membrane-bound APX (mAPX), and cytosolic APX (cAPX). As a first step towards the study of the gene regulation of the members of the Apx gene family, chloroplastic (Br-chlApx) and cytosolic (Br-cApx) isoforms transcript were isolated by RT-PCR in *Brassica rapa.* To investigate the changes of BrApx expression level in harvested broccoli a semi-quantitative RT-PCR were performed in different tissues (layer, stalk and florets) at different days (0, 4 and 14 d). Overall, the layer was the tissue with a higher expression level of the all BrApx isoforms. Also at 0 d and after harvest (4 and 14 d) Br-chlApx transcript in the layer and floret did not change. Whereas, in the stalk all BrApx transcript, except stromal Apx isoform, decreased more after harvest. It is important quantify the ascorbate peroxidase activity in broccoli after harvest to asses the therm-life for a healthy diet.

This work has been supported by "Seconda Università degli Studi di Napoli", "Ministero dell'Università" e "Ministero della Ricerca Scientifica e tecnologica" of Italy (PRIN 2008S9T3KK_003), "Regione Campania, PSR 2007-2013 Misura 214 azione f2 progetto Agrigenet".

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Poster Communication Abstract – 8.27

METABOLITES AND PEROXIDASE ACTIVITIES IN *BRASSICA RAPA* L. CV. *SYLVESTRIS* DURING POSTHARVEST STORAGE

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Brassica rapa L. cv. Sylvestris, postharvest storage, peroxidases, ascorbic acid, tocopherols

Leafy vegetables at harvest are subjected to considerable stress due to the sudden disruption in energy, nutrient and hormone supplies, They suffers an oxidative stress that activate senescence processes involving physiological biochemical and molecular events that affect also their nutritional, nutraceutic and organoleptic properties. In this work we studied the postharvest changes occurring in the edible part *Brassica rapa* L. subp. sylvestris (friariello napoletano) buy studying the patterns of metabolites as carbohydrates (glucose, fructose, sucrose), protein, chlorophyll, tocopherols, ascorbic acid and glucosinolates as well as of peroxidase isoenzymes. The analyses were done in floret, stem and leaf blade in plants kept stored in different conditions. The experiments were done by collecting the top turnips at commercial maturity. At the harvest the broccoli were saved in plastic boxes at 20°C, 10°C, 4°C and at 4°C in modified atmosphere (O2 5%; CO2 2%; N2 93%) up to 20 days from the harvest. At sampling, the edible part were separated in florets, stem and leaf blade, freezed and powered in liquid nitrogen, and, subsequently, used for the analyses.

During storage a significant decrease of carbohydrates (glucose, fructose, sucrose), protein, chlorophyll, tocopherols, ascorbate and glucosinolates occurred even if at different extent in all organs considered. Ascorbate concentration higher in leaf blades than the florets at the harvest, rapidly decreased in all organs during storage. Protein degradation was also accompanied by an increase in free amino acids. The peroxidase activities were due to acid, neutral or basic isoforms differently distributed in the plant tissues. The neutral and basic isoforms were dominant in leaf blade and florets, whereas acid isoforms were dominant in the stem. The activities increased significantly in florets and leaf blades during storage, mainly basic and neutral isoforms. In the stem, instead the activity decreased mainly for the decrease of the acid isoforms.

The storage at 4 °C, as expected, slow down the changes even if ascorbate concentration stayed higher in the samples kept in modified atmosphere storage.

The overall detailed results were analysed and discussed.

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Poster Communication Abstract – 8.28

NOVEL INSIGHTS AND PERSPECTIVES FOR THE BREEDING AND EXPLOITMENT OF HIGH-ANTHOCYANIN TOMATOES

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Anthocyanin mutants, antioxidants, breeding, nutritional value, Solanum lycopersicum L.

Because of their antioxidant activity, anthocyanins are considered important phytonutrients which contribute to the prevention of neoplasias, diabetes, coronary diseases and aging. The production of anthocyanins in the tomato fruit is absent or poor, but a number of natural or induced mutants are known to increase anthocyanin levels in vegetative tissues and fruits, like the photomorphogenic *high-pigment* (*hp*) and *hp-2* mutants, which increase the level secondary metabolites and thus the tomato nutritional quality. Furthermore, some tomato-related wild species produce anthocyanins in both plant and fruit, so that their *Abg, Aft, Aft^{ps}, atv* alleles, exhibiting varying degrees of anthocyanin pigmentation in the fruit epidermis, were introgressed into the cultivated tomato. A breeding activity carried out by our research group, aimed at combining different alleles controlling the anthocyanin production, recently obtained the *Aft_atvatv* combination, that showed the remarkable phenotype of a deep purple pigmentation of the pericarp, due to an increased level of anthocyanins in the fruit epidermis. Selecting for few generations led to a stable line that was characterized by good agronomic traits and a deeply purple colour of the fruit. Such line was called "Sun Black^{TM}", with reference to the importance of solar radiation for its expressivity. Due to the genetic control of the trait and to its expression in developing fruits, breeding of the Sun Black^{TM} phenotype in other backgrounds is cumbersome and costly. For this reason, we explored the possibility of using early-expressed markers to select precociously in segregating progenies the presence of one or more mutations affecting the anthocyanin pathway. To this aim, the intensity of the anthocyanin pigmentation was studied in roots and hypocotyls of seedlings at cotiledonary stage, by applying to different genotypes carrying anthocyanin-related alleles different dark/light combinations and supply of sucrose or phytormones. The amount of anthocyanins was higher in Sun Black^{TM}, especially if in combination with the *hp-2* allele. Root pigmentation resulted the best selection index for selection. Given the appreciable amount of anthocyanins formed by roots (where they are the only pigments present) in the most inductive conditions and the easy management of seedling production, the system could be susceptible of scaling-up to produce anthocyanins extracts in relatively reduced space and short time and target cosmetic, pharmaceutical or photovoltaic applications. Finally, further characterization of the Sun Black^{TM} line revealed other traits deserving agronomic interest, such as a fruit set potential higher than the wild-type and an enhanced shelf life evident in small fruits.

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Poster Communication Abstract – 8.29

DNA TESTING AS A MEANS TO PROTECT 'SAN MARZANO' PDO PRODUCTS

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SSR, blind analysis, 'San Marzano', peeled tomato, traceability

The tomato is one of the most frequently consumed vegetable world-wide and its production is largely based on hybrid varieties. However, some traditional cultivars play a significant role in the world market. One example is the 'San Marzano', a well-known local variety whose production is defended by an EU Protected Designation of Origin (PDO) label.

In the food sector, there have been cases that involved the substitution of a premium product with a less expensive or less desirable item, suggesting the possibility of deliberate mislabelling for economic gain. The present study shows that SSR markers can identify products of the tomato-food chain but also reveal mislabelling of commercial products.

We firstly used ten SSR markers to discriminate and seven blind coded lots of tomato berries, allowing the identification of five samples. Furthermore we also analysed commercial peeled tomatoes that were labelled as 'San Marzano'. Out of the ten SSR employed, seven successfully amplified fragments smaller than 200 bp from DNA isolated from tomato products. The allelic profiles obtained from the peeled tomatoes labelled as 'San Marzano' did not match the profiles of the 'Kiros', 'San Marzano 2' or of other genetically close accessions that can be used for the PDO production. Thus, molecular fingerprinting indicated that it is possible to exclude the presence of 'San Marzano' fruits in the analysed commercial products.

We demonstrated that selected SSR markers are a useful tool to protect the value of products entering and exiting the tomato food chain, as they are able to reveal mislabelling in commercial products.

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Poster Communication Abstract – 8.30

MOLECULAR CHARACTERIZATION AND DISCRIMINATION OF ITALIAN TOMATO CULTIVARS

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Solanum lycopersicum, tomato, SSR, genetic analysis

The tomato, *Solanum lycopersicum* (L.), is an autogamous species with a narrow genetic base. The introduction of the species in Europe, from Mexico, was crucial in the reduction of genetic variability, since in the European habitat tomatoes were generally cultivated in protected environments.

The high degree of genetic uniformity in tomato cultivars is not only strongly influenced by domestication far from the center of origin, but above all by genetic improvement which, *per se*, culminated in the achievement of uniform forms, apart from the fact that only a limited number of genotypes were used for breeding.

Tomato is nowadays one of the most economically important and widely grown plants in *Solanaceae* family. The popularity of tomato as fresh and processed crop has made it an important source of vitamin A and C in diets. In addition to its worldwide agricultural and economic importance as a crop, tomato is a preeminent model system for genetic studies in plants.

Simple sequence repeat (SSR) markers can be useful in variety identification and to analyze the relationships among cultivars. SSR analyses were conducted on four tomatoes sauces and 51 modern and vintage cultivated tomato cultivars, using eleven selected SSR primers. Cluster analysis allowed us to distinguish tomato in two major groups, the first included most of the fresh market varieties and the second included most of the modern processing varieties. The analysis showed a significant variation among varieties. The SSR approach showed considerable potential for tomato variety identification and discrimination. DNA profiles of these crops are even useful for quality control in agri-food chain, considering the possibility to identify the varieties present in the processed tomato.

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Poster Communication Abstract – 8.31

EVOLUTION OF SOME QUALITY PARAMETERS DURING FRUIT RIPENING IN *SOLANUM MELONGENA* L. INTROGRESSION LINES

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Chlorogenic acid, anthocyanins, glycoalkaloids, phenological stages

The contents of some nutraceutical and health-promoting fruit compounds were evaluated during ripening in innovative eggplant (*S. melongena* L.) introgression lines, their allied and cultivated parents. Such new genotypes, tolerant and/or resistant to the tracheomycotic fungi *Verticillium dahliae* Kleb. and *Fusarium oxysporum* f. sp. *melongenae*, respectively, were obtained from conventional and non-conventional breeding methodologies (i.e., sexual and somatic interspecific hybridization, androgenesis and backcrosses). The levels of chlorogenic acid, anthocyanins (nasunin and/or delphinidin 3-rutinoside) and glycoalkaloids (solamargine and solasonine) were studied in the flesh and peel of fruits at three phenological stages of ripening in 73 eggplant advanced introgression lines (ILs), 3 eggplant recurrent genotypes and 3 allied species (*S. sodomaeum*, *S. aethiopicum* gr. *gilo* and *S. aethiopicum* gr. *aculeatum*=*S. integrifolium*) during three successive years. Almost all the ILs, derived from several backcrosses cycles, evidenced some positive characteristics compared with the allied parents. Good levels of chlorogenic acid and anthocyanins and, mainly, significantly ($p \leq 0.05$) reduced concentrations of the toxic steroidal glycoalkaloids (SGAs) were detected in the ILs, specially in immature and commercially ripening fruit. In a few ILs, the safety limit (200 mg/100 g of dry weight) of SGAs was often exceeded in physiological ripening fruit; moreover, a marked reduction of chlorogenic acid and peel anthocyanins levels was also evidenced in this fruit stage.

These results confirmed the possibility to obtain new eggplant genotypes characterized by the tolerance/resistance against some tracheomycotic fungi coupled with interesting quality traits.

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Poster Communication Abstract – 8.32

RESOLVING GENE NETWORK THAT CONTROLS PHENOLICS ACCUMULATION IN TOMATO FRUIT

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Phenolics, antioxidants, tomato fruit quality, Solanum pennellii introgression lines, transcriptomics

Phenolic compounds are plant secondary metabolites that are important determinants in both sensory and nutritional quality of fruits and vegetables. In addition, in the last few years many studies studies support the increasing evidence of the possible role of phenolic compounds in prevention of chronic diseases such as cardiovascular disease and cancer [Nakajima et al., 2007, Life Sciences 80: 370–377]. Tomatoes are one of the valuable sources of antioxidants such as phenolic compounds (flavonoids and hydroxycinnamic acid derivatives). Therefore, increasing phenolics in tomato fruit is a major claim of breeding in order to meet consumer requirements and create new market opportunities. So far, many key genes have been reported that control the accumulation *in planta* of phenolic compounds. However, additional insights are required in order to highlight genetic and physiological mechanisms controlling phenols accumulation in tomato fruit and to support breeding programs for increased fruit quality. The aim of the work was the identification of major genes and gene networks that regulate the level of phenols in tomato fruit.

The screening of *Solanum pennellii x S. lycopersicum* introgression lines (ILs) [Eshed and Zamir, 1995, Genetics 141:1147-1162] over three year trials in greenhouse and open field environments allowed the identification of a stable QTL for increased fruit content of total phenolics in the IL7-3. In particular, chlorogenic acid mainly accounted for the higher performance of this line. Therefore, to investigate candidate genes controlling phenols synthesis and accumulation in IL7-3 fruit, we performed a comparative transcriptomic analysis in tomato pericarp between this line and the control *cv*. M82 over two consecutive years. The transcriptomic approach allowed to identify 149 up-rgulated and 142 down-regulated probes. Based on functional annotation, clustering and networking outputs, subsets of differentially expressed transcripts were used to develop model networks that describe mechanisms controlling accumulation of phenylpropanoids in tomato fruit. The network explains the variation in phenols levels in terms of interactions between ethylene signalling, plant responses to stress and biosynthesis of phenolics. Upon validation of key transcripts of our model by RT-qPCR we undertook a functional characterization of candidate genes by the TILLING (Targeting Induced Local Lesion IN Genomes) approach [Minoia et al., 2010, BMC Research Notes 3:69]. In particular, tomato plants homozygous for mutations in the coding sequence of two transcription factors involved in ethylene response (e.g. ERF1 and EIN3) revealed to drive ripening-associated phenol accumulation in fruit. These results suggested us to design new strategies of precision breeding for increased fruit nutritional quality in tomato.

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Poster Communication Abstract – 8.33

DETERMINING RESISTANCE TO *PSEUDOMONAS SYRINGAE* IN TOMATO, A COMPARISON WITH DIFFERENT MOLECULAR MARKERS

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Solanum lycopersicum, Pseudomonas syringae pv. tomato (Pst), Marker Assisted selection, Duplex PCR, HRM

Pseudomonas syringae pv. tomato (*Pst*) is the causal agent of bacterial speck disease in tomato. Resistance to *Pst* is determined by *Pto* a single resistance gene, that belongs to a multi gene family clustered on chromosome 5. *Pst* resistant phenotypes in cultivated tomato are determined by a semi-dominant allele of *Solanum pimpinellifolium*, which was introgressed into *Solanum lycopersicum* in the past century. Seed companies which are continuously interested in constituting resistant varieties, can benefit from genetic markers tightly linked to the *Pto* locus in breeding programme based on marker assisted selection. In this research three SCAR (Sequence Characterised Amplified Region) markers have been developed for the identification of resistant and susceptible genotypes of *Solanum lycopersicum*. A CAPS marker has been adapted to a real-time PCR platform with an High Resolution Melting analysis. Application to a segregating population is described. Pros and cons of the different markers are discussed.

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Poster Communication Abstract – 8.34

ANTIOXIDANT CONTENT IN TOMATO VARIETIES AND ECOTYPES

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Ascorbic acid, polyphenols

Secondary metabolites play an important role in plant metabolism although they are produced in a low amount. Among secondary metabolites, antioxidants such as ascorbic acid and polyphenols provide selective advantages such as weeds control or resistance towards several pathogens. Tomato (*Solanum lycopersicum*) is widely cultivated all over the world for food purpose and in addition it is rich in polyphenols and ascorbic acid.

The final purpose of this study is pointing out the genetic control mechanisms underlying different antioxidant accumulation patterns therefore we began to evaluate ascorbic acid and polyphenol accumulation in leaves of different tomato genotype. Particularly, we have focused on the following Italian genotypes: Ventura Fiaschetto selection, Belmonte, San Marzano Nano, Principe Borghese, Tondino Maremmano and Ponderosa (kindly provided by SemiOrto Sementi, http://www.semiorto.com), and Piennolo, Tondo Giallo and Maggese (from local farmers of Campania).

Our results showed that total ascorbic acid (reduced plus oxidized forms) is differently accumulated in tomato genotypes. The lowest amount was in Tondino Maremmano variety which, in contrast, showed to accumulate the highest total polyphenol amount than the others. Among genotypes tomato Ponderosa variety showed to accumulate a high amount of both antioxidants.

In conclusion, ascorbic acid and polyphenol accumulation in tomato leaf is genotype dependent. Furthermore, efforts will focus on ability of these varieties to contrast or tolerate biotic stresses, and through transcriptomic approach to add insights toward strategies for breeding tomato resistance and quality.