MOLECULAR TRACEABILITY EXPLOITING A GRID-ENABLED WORKFLOW ENVIRONMENT


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molecular traceability, qPCR, workflow development

The composition of a specific food is a key factor in determining the quality and the safety of the product. The reliable identification of plant, animal and microbial species is therefore essential for handling, marketing and processing of grain and derived products.

Usually, the traceability lab working flow starts with the identification of a set of DNA sequences publicly available on reference databases and selected via a functional annotation based query.

On the basis of the researcher knowledge, a deeper selection of the hypothetical optimal sequences is then obtained. The chosen sequences are subsequently aligned by means of a dedicated, locally installed or web based, software. In this way, common DNA regions are underlined and a more restricted set of sequences is selected for the lab analysis.

As a consequence of the multi-step nature of the analysis, involving several “in silico” activities devoted to sequence selection, the possibility of building up customizable workflows can be of great help in the traceability process. Moreover, ring tests are often necessary for result validation, leading several research groups to work in a collaborative manner.

Solutions for these needs can come from the MiPAF founded project named “ESCOGITARE” which is aimed at applying e-Science and the related technologies to support collaboration among the different institutes of the Italian Agricultural Research Council. One of the main target of the infrastructure developed within ESCOGITARE is to enable experiments involving different labs spread all over Italy and to use a workflow management system for the selection, in a transparent and secure way, of data and instruments installed in several locations.
GENOTYPIC IDENTIFICATION OF A **DEBARYOMYCES HANSENII** YEAST STRAIN ISOLATED FROM TOBACCO LEAVES


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*Debaryomyces*, genotypic identification, phyllosphere yeasts, tobacco leaves, yoghurt

The surface of plants accommodates a varied microflora. This environment is usually named the phyllosphere (Middelhoven, 1997). Yeasts play an important part in this microflora. Little is known of the biochemical activity of yeasts growing on plants. Growth at the expenses of sugars and other nutrients leaking from the plant during rain storms or dew precipitation is supposed. Mechanical damage of the cuticle and cell walls also result in leakage of nutrients (Middelhoven, 1997).

We are involved in the isolation of yeasts relevant to dairy product manufacture. The outline of the present study is isolation and genotypic identification of the yeast present in tobacco leaves growing in Campania Region. Air-dried tobacco leaves were eluted in sterile distilled water (s.d. H₂O), overnight at 4°C. After centrifugation in Sorvall at 8,000 rpm for 10 min., the pellet was resuspended in 5-10 ml s.d. H₂O. Dilutions of that microorganism cell suspensions were plated on MM102 agar medium (0.4g NH₄Cl, 0.2g NaCl, 0.16g MgSO₄·7H₂O, 0.06g KH₂PO₄, 0.14 K₂HPO₄, 0.4 CaCO₃, 1g Glucose, 22g bacto agar and H₂O to 1l).

After 4-5 days incubation at 30°C single colonies were eluted in s.d. H₂O and yeast cells were observed by microscope. Purification of the yeast strain was performed on the same medium by successive subculturing.

The taxonomical position of *Debaryomyces hansenii* DYCR-29 yeast strain isolate with respect to related strains was determined in this study by nucleotide sequencing of the 18S rRNA gene and the ITS1-5.8S rRNA-ITS2 region. On the basis of the available sequences in the database, the variety *hansenii* was the closed related to our yeast.

A range of observations indicate an ability of yeasts to metabolize milk constituents. These observations include the occurrence and growth of yeasts in many cheeses, especially soft cheeses and yoghurts (Roostita and Fleet, 1996).

*Debaryomyces hansenii* was the predominant yeast species most frequently isolated from ripened cheese. This yeast impact on cheese quality and flavour through their production of lipolytic and proteolytic enzymes, fermentation of residual lactose, utilization of lactic acid and their autolysis (Ferreira and Viljoen, 2003). Work is in progress for utilizing the *Debaryomyces hansenii* DYCR-29 strain as co-starter in yoghurt production.


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AGRONOMICAL, TECHNOLOGICAL AND SENSORIAL EVALUATION OF NEW AND OLD WHEAT VARIETIES FOR THE PREPARATION OF A TRADITIONAL ITALIAN SOURDOUGH BREAD

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sourdough bread, old and new bread wheat varieties, sensory evaluation, traceability

The purpose of this project has been to evaluate a panel of old and modern Italian bread wheat varieties for the production of a sourdough bread traditionally prepared in a hill zone of the North of Italy.

The agronomic performance of the varieties has been evaluated in different environments, both in conventional and organic farming systems, and technological properties of the grain and of the flour have been determined.

Sourdough bread was prepared from the flour of the different varieties grown both in conventional and farming system and sensory evaluations were performed.

The microbial population of the sourdough was characterized with molecular approaches.

A traceability system based on the use of molecular markers like SSR has been developed to certificate authenticity of this traditional bread in term of bread wheat varieties used for its preparation.
PUROINDOLINES IN BREAD WHEAT NEAR-ISOGENIC-LINES (NILs) OF CV. ENESCO


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puroindolines, hardness, bread wheat, Near-Isogenic-Lines

Kernel hardness, an important technological trait influencing flour yield and quality of common wheat, is under the genetic control of \( \text{Pin}_{a}\)-1 and \( \text{Pin}_{b}\)-1 loci coding for puroindoline a (Pin-a) and puroindoline b (Pin-b), respectively. Pin-a and Pin-b are the predominant component of friabilin, a group of polypeptides which occur in higher amounts on the surface of starch granules of soft wheats as compared to those from hard wheats.

The main common wheat cvs grown in Italy have been assigned to three different hardness classes (soft, medium hard and hard) according to their SKCS values. All cvs in each hardness class were found to show a normal distribution for the SKCS index, with the only exception of cv. Enesco, which presented a bimodal distribution. Further investigations carried out on spaced plants from certified seed of cv. Enesco demonstrated that plants were similar in phenotype and prolamin pattern, but different in puroindoline composition. Two biotypes were identified, i.e. soft textured Enesco 1 possessing alleles \( \text{Pin}_{a}\)-1a and \( \text{Pin}_{b}\)-1a and hard-textured Enesco 2 possessing \( \text{Pin}_{a}\)-1b and \( \text{Pin}_{b}\)-1a. The commercial cv. Enesco and its biotypes have been used in a field experiment carried out in Rome in 2006 and repeated in 2007 at S. Angelo Lodigiano (LO) in a plot trial with randomized blocks.

The tested genotypes were found to be similar for grain yield and agronomic related traits and significantly different for quality aspects such as flour yield, water absorption, starch damage, farinograph time peak and stability, alveograph W and P/L. Different end uses for each biotype are suggested according to their puroindoline composition.
DISCOVERY AND CHARACTERISATION OF PUROINDOLINE GENES IN WILD TETRAPLOID AND HEXAPLOID WHEATS (TRITICUM ARARATICUM, T. TIMOPHEEVII AND T. ZHUKOWSKY)

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grain hardness, puroindolines, Triticeae, wild wheats

Differences in grain texture have proved to play a large impact on end-product quality of cereals, contributing to the distinction of well suited market classes of endosperm end-uses. Variation in texture is associated with the presence/absence or sequence polymorphism of puroindoline a and b, i.e. the products of the Ha locus, located on the short arm of chromosome 5D. In search for new genetic resources of grain hardness, several alleles have been recently identified in a large amount of cultivated and wild wheats, including A, B, D, C and U diploids, barley and rye. Nevertheless, AB tetraploid wheats have repeatedly shown to be devoid of puroindolines, whereas other major polyploid lineages of Trititectae have been little inspected and with contrasting results. In this paper we report the discovery and characterisation of puroindoline a and b genes in some wild tetraploid and hexaploid wheats [Triticum araraticum (AAGG), T. timopheevii (AAGG), and T. zhukowsky (AAAAGG)], where the absence of the Ha locus had been previously postulated due to unsuccessful isolation of the sequences.

Nucleotide sequence comparisons with wild types from cultivated wheats and with alleles detected in other wheat genome bearers displayed various degrees of insertions/deletions and point mutations. Despite some amino acids substitutions, the deduced main features of the proteins secondary structure were all mantained. Data on the proteins expression are also reported and discussed.

In addition to providing insights on the evolution of these genes in Triticeae, and on the genome group relationships, our data enlarge the current knowledge on puroindolines molecular system and contribute to further inspect their potential use for the genetic improvement of wheat.
EXPRESSION OF LIPOXIGENASE GENES DURING GRAIN-FILLING PERIOD IN DURUM WHEAT

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lipoxygenase, gene expression, semi-quantitative RT-PCR, grain filling period, durum wheat,

The large reduction of natural carotenoid during pasta processing is mainly due to oxidative degradation by lipoxygenase (LOX) activity. LOXs are iron non-heme, containing dioxygenases that catalyze the oxidation of polyunsaturated fatty acids (e.g. linoleic and linolenic acids) in plants, animals and microorganisms. On the basis of a previous screening work in the present study 4 selected durum wheat (Triticum durum Desf.) cultivars, contrasting for endogenous LOX activity: cv. Primadur and Trinakria with high LOX activity and cv. Cosmodur and Creso with low LOX activity, were used to evaluate the activity and the expression profile of the LOX genes during the grain filling period (GFP). Different primer combinations based on published sequences were designed to amplify specifically the durum wheat sequences putative orthologous to the barley LOXA, LOXB, or LOXC genes. LOX showed two distinct peaks of activity. The first one occurred in the early stages of grain development (8 DAF), whereas the second one occurred at the later stages (30 DAF). Semi-quantitative RT-PCR was conducted on the cDNA template synthesized from mRNA on whole developing durum wheat grains from 8 days after flowering (DAF) to full maturity. The temporal expression of three LOX genes showed a different profile among the cultivars evaluated. Trinakria and Primadur (high LOX activity on wholemeal at maturity) showed an anticipated temporal expression for LOXA compared to Creso and Cosmodur (low LOX activity). The latter cultivars also showed a lower gene expression for LOXB at the later stages (30 DAF) respect to Trinakria and Primadur. LOXC gene, characterised by an high expression level in the early stages, did not show any significant differences in mRNA levels during GFP among all evaluated cultivars. These results confirm the previous finding on barley suggesting the existence also in durum wheat of three putative LOX isoenzymes (LOX1, LOX2 and LOX3). Cloning, expression, and functional analysis of genes coding for LOXs are ongoing for understanding their physiological role in durum wheat.
COMMON WHEAT DETECTION IN DURUM WHEAT SEMOLINA BY MICROSATERNLITE-BASED REAL-TIME PCR

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durum wheat semolina, DNA microsatellites, common wheat detection, polymerase chain reaction

The detection of common wheat (Triticum aestivum L.) contaminations in durum wheat (Triticum turgidum L. var. durum) semolina has always been the object of interest and stimulated the development of numerous analytical methods, generally by searching specific common wheat protein fractions. These methods were mainly aimed to preserve pasta “purity” and check its adherence to Italian rules. Recently, the typical Altamura bread (Apulia, Italy), has been awarded with the European Protected Designation of Origin (PDO) mark, owing to its typicality. This product should be prepared using only durum wheat semolina, and should not contain soft wheat flour. The aim of this work was to evaluate the possibility to apply DNA microsatellite analysis to set up a method for the detection of soft wheat in durum wheat semolina. This strategy can be applied for checking the raw materials used for Altamura bread, as well as for Italian pasta preparation. A total number of 9 primer pairs amplifying microsatellite sequences was chosen for being localized on D-genome according to literature data, and was tested on the DNA extracted from semolina and flour of various durum wheat and soft wheat cultivars, with the aim of verifying the effectiveness in distinguishing common wheat from durum one. The obtained results allowed to select, among them, an efficient D-genome-specific microsatellite. SYBR Green real-time PCR enabled to successfully detect common wheat in semolina, with a threshold of 2.5%.
A STRATEGY FOR INCREASING AMYLOSE CONTENT IN DURUM WHEAT BY THE OVEREXPRESSION OF A WAXY GENE

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starch, wheat, waxy, high-amylose

Reserve starch provides a food energy source for the human population. It is composed of two different glucan polymers: amylose and amylopectin. Amylose is a linear polymer and it constitutes about a third part of total starch, whereas amylopectin results in a branched structure and constitutes the remaining starch. The waxy protein is a granule-bound starch synthase responsible for amylose synthesis; in durum wheat (genomic formula AABB) two different isoforms are present which are encoded by two genes designated as \( Wx-A1 \) and \( Wx-B1 \) located on chromosome arms 7AS and 4AL respectively.

The amylose/amylopectin ratio influences the physical-chemical properties of starches. By altering the regulation of starch biosynthesis, it is possible to modify the amounts of amylose and amylopectin and to produce starches with new unique properties. High amylose starches are particularly interesting because they have an increased content of resistant starch that has beneficial effects on human health. The nutritionists believe that the resistant starch has a role similar to fibres inside the intestine preventing diseases as colon cancer, diabetes and obesity.

In order to produce transgenic wheat lines with high-amylose starch, the \( Wx-B1 \) gene has been isolated by RT-PCR from immature (21 days after anthesis) wheat kernels of the durum wheat cultivar Svevo and cloned in a vector for biolistic transformation. The construct contains an endosperm-specific high-molecular-weight glutenin promoter. Immature embryos of two durum wheat genotypes: Svevo and Svevo waxy (low amylose mutant lacking both \( Wx-A1 \) and \( Wx-B1 \) proteins) have been transformed. Eighteen transgenic lines have been regenerated (5 lines from Svevo and 13 lines from Svevo waxy) and screened by PCR using primer pairs specific for the construct. The presence of the transgene has been confirmed also by Southern blot analysis. To investigate if the \( Wx-B1 \) protein was overexpressed in putative positive plants, SDS-PAGE and densitometric analysis has been performed on the starch granule-bound proteins.

From our data it results that only two lines (generated by transformation of Svevo) overexpress the waxy protein. The analysis of the Svevo waxy transgenic has shown that some lines restored the wild type phenotype, although the \( Wx-B1 \) protein has not been found on the granules.
CHARACTERIZATION OF NEW MUTATIONS OF WAXY GENES IN BREAD WHEAT

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Starch, wheat, waxy, amylose

Starch is composed of two kinds of glucan chains, one highly branched called amylopectin, the other one essentially linear called amylose. Starch biosynthesis takes place within amyloplasts and involves the concerted action of different enzymes. Two different pools of enzymes are responsible for the synthesis of these two polymers; the granule bound starch synthases (GBSSI) or waxy proteins elongate the amylose chains, whereas starch synthases, branching and debranching enzymes cooperate to the formation of the complex amylopectin structure. In hexaploid wheat three isoforms of waxy proteins are present, encoded by three genes designed as Wx-A1, Wx-B1 and Wx-D1, respectively located on chromosome arms 7AS, 4AL and 7DS.

By electrophoretic analyses we have been able to identify new waxy null mutants in several hexaploid wheat accessions from worldwide collections and investigated the molecular basis responsible for waxy gene silencing in the different mutants identified. The molecular characterization of mutated waxy genes in four bread wheat cultivars (two lacking the Wx-B1 and two lacking the Wx-A1 protein, respectively) has been carried out by means of PCR and DNA sequence analyses.

In order to determine the presence of polymorphisms in these new mutants, several genome-specific primer pairs have been designed for the Wx-A1 and Wx-B1 genes.

By these analyses we have found that two complete gene deletions are responsible for the null alleles at the Wx-A1 and Wx-B1 loci in two different bread wheat lines. In a third bread wheat line the presence of a 18 bp deletion at the splicing site between the second exon and second intron in the Wx-A1 locus seems to be responsible for the absence of the Wx-A1 protein.

The cause of the absence of the Wx-B1 protein in the fourth bread wheat analyzed was not determined. In fact, though nucleotide substitutions and single-nucleotide insertions/deletions were detected in the PCR fragments analyzed, the deduced amino acid analysis revealed that the observed mutations not produced frameshifts with the insertion of a stop codon.
PRODUCTION OF DURUM AND BREAD WHEAT LINES WITH HIGH AMYLOSE STARCH

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starch, wheat, SGP-1, starch synthase, amylose

Starch is the predominant carbohydrate reserve of wheat grain and accounts for 65-75% of grain dry weight in the wheat endosperm and it is composed of two different glucan chains, amylose and amylopectin. The relative amounts of amylose and amylopectin, which in normal wheat starch vary from 1:3 to 1:5, are responsible for its unique physical and chemical properties with strong influences on functional properties of flour or semolina and on its specific uses in the food and manufacturing industries. The enzymes directly involved in amylopectin biosynthesis are starch synthases (SSs) and starch branching enzymes (BEs). The waxy protein is a granule-bound starch synthase responsible for amylose synthesis. In this work we have focused on starch granule protein-1 (starch synthase II or SSII). In hexaploid bread wheat (AABBDD) three different isoforms are present which are encoded by three genes designated as SGP-A1, SGP-B1 and SGP-D1 located, respectively, on chromosome arms 7AS, 7BL and 7DS. In tetraploid durum wheat (AABB) two isoforms are present, associated to the SGP-A1 and SGP-B1 loci.

Partial Sgp-1 mutant lines lacking one of the three possible SGP-1 proteins have been identified by Yamamori et al. (2000) through an extensive electrophoretic analysis. Crossing of these materials has permitted the combination of different null alleles detected both in a bread wheat line (N11) and in a durum wheat cultivar (Svevo) with the production of the entire set of partial lines along with the complete SGP-1 nulls. These lines show a high amylose content (30-40%) and contain slightly increased levels of resistant starch. The resistant starch has a role similar to dietary fibres and it has numerous beneficial effect on human health. SDS-PAGE experiments reveal that the levels of two starch granule proteins, SGP-2 (starch branching enzyme) and SGP-3 (starch synthase I enzyme), decrease considerably in the SGP-1 null wheat, whereas the waxy proteins remain unaltered. To investigate if there are pleiotropic effects on SGP-2 and SGP-3 genes in total SGP-1 mutants, specific primers have been identified and used for RT-PCR analyses on wheat kernels collected at different development stages.

The effects of the different SGP-1 mutations on starch-pasting properties have been assessed by RVA (Rapid Visco Analyzer) analysis.

Twelve genome specific primers for SGP-1 alleles have been identified and can be used for indentifying new natural or induced mutants by PCR or TILLING approaches or as molecular markers to follow SGP-1 introgression in superior wheat genotypes.
FUNCTIONAL MARKERS FOR THE IMPROVEMENT OF RICE GRAIN QUALITY


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aroma, starch quality, MAS, Oryza sativa ssp.japonica

Rice serves as a staple food for about half of the world’s people: due to its strategic importance as human food, rice is extensively studied for improving both yield and quality. Improvement of rice quality – either as simple rice or derivatives - is a major objective in current breeding programs, especially in relation to cooking quality. High amylose rice and aromatic rice are two important classes of products for the rice milling industry. Aroma and amylose content are two main determinants of rice quality: the present study is addressed to the exploitation of molecular markers for breeding programmes of aromatic and amylose differentially containing rice varieties.

A series of Italian modern varieties and exotic varieties currently used in Italian rice breeding programmes have been analyzed by means of microsatellite (SSR) molecular markers in aid to traceability of the particular traits involved in determining their quality.

Fragrant rices have in recent years gained wider acceptance also in Europe. The most important component of fragrance in Basmati and Jasmine varieties is the chemical compound 2-acetyl-1-pyrroline (AP). The accumulation of 2-AP in aromatic rice is determined by a recessive trait; it has been shown to be due to an 8 bp deletion and three SNP’s in a gene which encodes a putative betaine aldehyde dehydrogenase (BAD2). The accumulation of AP in fragrant rice varieties may be explained by the presence of mutation resulting in a loss of function of the fgr gene product. A series of newly developed varieties and lines have been characterized by means of fgr SSR markers. A SSR marker for the recessive trait has been identified and demonstrated to be a useful tool during early selection. Starch is composed of amylose and amyllopectin, and apparent amylose content (AAC) has been well recognized as one of the most important determinants of various rice product. Amylose content in rice spans from nearly zero (glutinous rice) up to 30% (high amylose rice). The amylose content is primally controlled by the waxy gene. Two functional markers- a (CT)n microsatellite and a G/T single nucleotide polymorphism (SNP) - have been well characterized with different alleles differing in AAC, and currently used as aid in selection (MAS).

The development of the SSR profiles will help breeding programmes to identify the genetic determinants of important quality traits, and use them in the constitution of competitive new varieties. The study is financially supported by the project VALORYZA (MIPAAF - DM 301/7303/06).
Glycoalkaloids are potentially toxic compounds found especially in plants from the Solanaceae family. There are many different glycoalkaloids produced by the various members of this family. Cultivated and wild potato species synthesize a wide variety of steroidal glycoalkaloids (GA). The toxicity of potato glycoalkaloids is far greater in man than for other animals studied, with levels of between 3 and 6 mg/kg being reported as lethal, levels comparable to that of strychnine (5 mg/kg). During breeding programs, species genomes are often put together through either sexual or somatic hybridization. Therefore, the determination of the GA composition of hybrids is very important in that it may affect either human consumption or resistance to pathogen and pests. This work reports the results of glycoalkaloids (GA) analysis performed on wild Solanum bulbocastanum, haploids of cultivated potato S. tuberosum and their interspecific somatic hybrids. Glycoalkaloids were extracted from tubers and analysed by HPLC. S. tuberosum haploids HPLC profile showed, as expected, the presence of α-solanine and α-chaconine. The profile of S. bulbocastanum extract showed lack of α-solanine and α-chaconine and the presence of four GA (not yet identified). The GA pattern of the somatic hybrids was the sum of their parents’ profile. This represents a noteworthy tool for their unequivocally recognition. Interestingly, two hybrids produced not only glycoalkaloids of both parents but also probable new GA to be further investigated. This provided evidence that somatic hybridization induced the synthesis of new metabolites. The nature of the probable unidentified glycoalkaloids associated to S. bulbocastanum and its somatic hybrids was ascertained by chemical degradation and spectroscopic analysis of their aglycones and sugar moieties. Our results suggest their close relation with glycoalkaloids of both wild and cultivated potato species.
ANALYSIS OF THE GENETIC DIVERSITY IN PROMOTER SEQUENCES OF GENES ENCODING FLAVONOID RELATED ENZYMES IN STRAWBERRY

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strawberry fruit, flavonoid biosynthesis, gene expression, genetic diversity

Flavonoids are secondary metabolites involved in several biological processes such as pigmentation, protection against UV radiation and pathogens, fertility and pollen germination. Cultivated strawberry (*Fragaria x ananassa*) accumulate large amounts of flavonoids, particularly proanthocyanidins and anthocyanins, during fruit development and ripening. The flavonoid accumulation pattern in strawberry fruits is highly dependent on genotype and environmental conditions.

We report a comparative characterization of the promoter regions and transcript levels of the principal structural genes involved in flavonoid biosynthesis in three genotypes previously selected for their different flavonoid accumulation pattern in ripe fruits.

Based on coding sequence data, we have performed upstream genomic walking in the promoter region of three flavonoid genes, and compared their sequences in the selected genotypes.

A very high sequence divergence was observed for leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR) gene promoters (up to five and four promoter types, respectively for LAR and ANR), while dihydroflavonol reductase (DFR) promoters showed a quite low sequence variability (to date, only one promoter form was found). Furthermore, we have developed a PCR-based screening to discriminate promoter-associated sequence polymorphisms among genotypes, some of which appear to be correlated with flavonoid accumulation in fruit.

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TRANSCRIPTOMIC ANALYSIS OF ACID AND NON ACID LEMON FRUITS


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citrus, acidity, microarray

Citrus fruits are characterized by the accumulation of high levels of citric acid, which account for 90% of the total organic acids. Citric acid is extremely important for the definition of the fruit taste because it affects the sourness of the fruit as well as the sweetness, by masking the taste of sugars. Many studies have tried to explain the different content of citric acid analyzing the fluxes through the enzymes of the TCA cycle or the transport of metabolites between cytosol and mitochondria. However the mechanisms of acids accumulation in lemon fruit are not yet well clarified.

To study the citric acid accumulation we compared the transcriptomes of juice sac cells in acid and acideless lemon genotypes. For the first time since its commercialization, the 30K Affymetrix Citrus GeneChip® was used. The sequence information for the development of the array was obtained from the Citrus HarvEST and cDNA clustering database, provided by Dr. Close and colleagues at UC Riverside.

Three genotypes were used for the transcriptome analysis: Frost Lisbon (FL), a standard sour lemon, and two Faris genotypes obtained from a chimera that produces on the same tree both sweet (Faris non acid, FLA) and sour fruit (Faris acid, FA). Transcriptome comparisons between the three genotypes during two ripening stages showed 4189 differentially expressed genes. Data mining on these genes has identified the close relation between TCA cycle substrates and amino acids metabolism suggesting that the expression of a Plasma type proton pump could be the cause of different pH between FNA and sour genotypes.

The poster will present these data and a new hypothesis on the mechanisms that are activated when the vacuolar acidification is blocked.
A USEFUL METHOD FOR THE IDENTIFICATION OF PLANT GENERA IN FRESH FRUIT JUICES

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traceability, fresh juices, rbcL, 5S rRNA

The aim of our study was to develop a method to trace fruits used for juice production in order to guarantee authenticity and protect the consumer against adulteration, either through accidental or fraudulent substitutions, that can lead to lower the value of high quality raw material.

The ability to extract DNA from fresh juices using a CTAB based protocol was tested in order to obtain genetic material suitable for PCR applications. Quality DNA extraction from juices may be difficult due to high polysaccharides and PCR inhibitors content, therefore, samples were extracted according to Tel – Zur. DNA was diluted and tested for PCR using a common primer pair developed by Ortola – Vidal and new primers designed in this study. The target sequence was a part of the large sub-unit of ribulose biphosphate carboxylase gene (rbcL), a conserved multi-copy plant gene containing single nucleotide polymorphisms able to discriminate between plant genera.

The study is also on the way of implementing a mini sequencing approach, designing species specific primers with the 3’ terminus upstream any specific single nucleotide mutation, able to distinguish among the genera Rubus, Fragaria, Vaccinium, Punica, Citrus, Ribes and Malus. Using the new designed primers on rbcL sequence the maximum amplicon length achievable from such a processed matrix was also determined.

Furthermore, with the purpose of obtaining a quantitative analysis, another method involving the amplification of 5S rRNA gene region using new primers was also developed. These primers were designed to amplify species-specific fragments distinguishable by means of a quantitative Real Time approach employing SYBR Green and recording of the melting curve.


APPLICATION OF FOOD GENOMICS TO OLIVE OIL TRACEABILITY

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food genomics, olive oil, SSR markers, DNA exctracation

Olive oil is one of the most important agricultural product of European Union. About three-quarters of global olive oil production comes from European Union member states; 77% of the European production comes from Spain, Italy, and Greece. The European Union occupies the first place in the world, with a production of 80% and a consumption of 70%. Since 1992, with the aim of protecting the typicalness of food products and discouraging similar products competition, European Commision has created certification labels known as Protected Designation of Origin (PDO) and Indication of Geographical Provenience (IPG) (EEC Regulations n. 2081/92). The introduction of quality labels has led to the need of controlling the product compliance to specific regulations about production.

To assess the geographical and varietal origin, fatty acids, triglycerides, sterols and general chemical composition were analyzed for olive oil. These parameters are often variable and they are influenced by environmental factors, while plant DNA sequence in olive oil should be independent from the environment, and it might be used to trace specific plant genotypes in this complex food matrix. Promising results come from the application of molecular markers, especially those based on PCR such as: Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs). In the present study we demonstrated that SSR markers are useful for the traceability of olive oils. SSRs are used in genetic analysis and in fingerprinting studies, they are characterized by an high degree of polymorphism and can be applied to high-throughput analytical systems.

At present DNA from olive oil has been extracted and analyzed with molecular markers by several research groups. To improve the applicability of SSRs to traceability of DNA extracted from olive oil, we performed a detailed evaluation of the entire methodology. Twenty one types of monovarietal oils were analyzed with nine nuclear microsatellite markers. We estimated for every marker the correspondence of allelic profile with reference cultivar, the reproducibility of profiles in different DNA extractions and the discrimination power of analytical system. The markers applied demonstrated different analysis efficiency depending on the matrix effect of each olive oil and on the different quality level of DNA extracted. To overcome the problem of degraded DNA we reduced the size of SSR fragments to below 150 bp using the mini-STR technology. Significantly better results were reported using this approach. We also evaluated the problem of paternal contribution in the analysis of olive oil produced by crushing whole olives. No significant differences were reported in analysis of DNA extracted from olive oils made from stoned and destoned olives.
APPLICATION OF FOOD GENOMICS TO WINE TRACEABILITY

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food genomics, wine traceability, SSR markers, DNA extraction

Wine is one of the most important agricultural products of European Union. Communitarian and national severe normatives regulate the production and commercialization of high quality wines marking them with certification labels. Now the search for methods to identify the cultivars used in wine making is the most important goal to ensure the origin and final quality of the products. Until now several analytical methods evaluating biochemical parameters were tried. The ratios of trace elements and isotopes were analyzed to assess the geographical origin while total grape must proteins in polyacrilamide gels (native-PAGE), phenolic and amino acid profiles, terpens and other aromatic compounds were used to assess the varietal origin. Promising results come from the application of molecular markers, especially those based on PCR such as: Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs). Current opinion of scientific community considers a bottle of wine like a forensic or ancient sample because the DNA is extremely degraded during technological process. In the present study we demonstrated that SSR markers are useful for the traceability of wines. SSRs are used in genetic analysis and in fingerprinting studies because they are characterized by an high degree of polymorphism and can be applied to high-throughput analytical systems.

DNA extraction from commercial wines has been reported in only two papers while PCR based molecular markers have been successfully applied to the analysis of grape juice, musts, fermenting musts and unprocessed wines. The experience of our laboratories, acquired in other fields, such as detection of GMOs in processed foods and olive oil traceability, allowed us to improve considerably the DNA extraction techniques from complex food matrices. We demonstrated that traces of *Vitis vinifera* DNA are present not only in unprocessed wines but also in bottles of processed and aged experimental and commercial wines. DNA extracted was amplified with a panel of six nuclear and two chloroplastic SSR markers and we evaluated the discrimination power, the reproducibility and the efficiency of each SSR marker and of the entire analytical system. Nuclear SSRs are significantly more polymorphic and have an higher discrimination power than chloroplastic microsatellites; however they are more hardly detectable in DNA extracted from commercial wines. On the other hand, chloroplastic genome is in high copy number and this is an advantage when DNA is in low amount and degraded but its variation degree is very low. To improve the tools for wine traceability different strategies are feasible: a) the optimization of DNA extraction methods, b) the reduction of the size of SSR fragments under 150 bp using the mini-STR technology, c) the use of SSR composed by tri-, tetra- and pentanucleotide repeats useful to reduce the problem of stutter products and to solve wine mixture analysis, and finally d) the increase of the number of chloroplastic markers. The future combination of SSR and SNP chloroplastic markers can be a good possibility to overcome the problem of the low degree of polymorphism. Therefore
chloroplastic markers can be useful for wine traceability, such as mitochondrial markers are useful for human forensic DNA analysis when nuclear DNA is degraded and in low copy number.