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DNA ANALYSIS OF OLIVE OILS FOR MOLECULAR TRACEABILITY

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In the last few years several protocols have been developed and implemented for extracting DNA from different food matrices. Moreover, several commercial kits based on proprietary purification techniques have been put forward by different companies. Nonetheless, protocols specifically designed for extraction of DNA from olive oil are still rare. We have therefore applied to olive oils, differing in their processing leve., a variety of methods, both commercial and taken from literature, modified and adapted from protocols originally designed for application to other food matrices, to recalcitrant plant material, and to different biological samples. In total, 26 protocols have been tested. They differed in the basic principle underlining the procedure: some are based on CTAB, whereas commercial kits are based on DNA-binding resins or particles; other methods are based on still different detergents and approaches. The parameters used to evaluate the success of a protocol for DNA extraction were the following: (i) DNA yield of the procedure, estimated with spectrophotometric or fluorimetric techniques; (ii) amplificability of the DNA obtained in different PCR reaction conditions; (iii) dimensions of the DNA fragments obtained; (iv) presence of olive DNA, estimated through amplification of species-specific genomic fragments. Methods based on CTAB buffers extract DNA from amounts of oil ranging from 6 to 400 ml. Most methods can yield DNA which can be amplified with chloroplastic primers and RAPDs. For methods based on DNA binding resins, starting volumes are very low, but yields can be considerably higher than those obtained with CTAB methods, up to micrograms per ml of oil. In general, most methods allowed amplification with universal primers and with olive-specific primers. Finally, some methods based on different approaches were developed in-house. An hexane-based protocol showed to be successful in different laboratories, yielding high quantities of DNA which could be amplified with all types of primers.A number of protocols tested for DNA extraction proved to be adaptable to different types of commercially available olive oils. Out of all, four protocols, are also applicable in an industrial setting: the amount of starting material, from few millilitres to a maximum of 160 millilitres, the intrinsic technical difficulties of the procedures, the equipments required, the labour needed and the cost are all compatible with a scaling up at industrial level. The DNA extracted with these methods is rather pure, the fragment size sufficiently large to be fingerprinted with the more specific molecular descriptors available for identity, distinctiveness and uniformity search and definition. This study has been carried out with financial support from the Commission of the European Communities, specific RTD programme "Quality of Life and Management of Living Resources", QLK1-CT-2002-02386, "Traceability of origin and authenticity of olive oil by combined genomic and metabolomic approaches (OLIV-TRACK)". It does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

OLIV-TRACK: GENOMIC AND METABOLOMIC APPROACHES FOR TRACEABILITY OF OLIVE OIL

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The OLIV-TRACK research project has the main objective of applying molecular technologies based on genomic and metabolic information to the traceability of origin and authenticity of olive oil produced and sold within the European Union. Traceability is not only relevant to assessing origin and content of the olive oil but also to protect and prevent frauds at different stages in oil production. For this purpose global metabolic information on olive oils obtained through mass spectrometry and other analytical procedures will be compared with the total genomic information obtained through molecular marker technology. The first Task performed by the Consortium has been the demonstration of the feasibility of DNA extraction from extra-virgin, virgin and refined olive oils. DNA extracted from oil could be amplified with primers targeting chloroplastic and nuclear genes, microsatellite loci and RAPD fragments. The same extraction methods have been applied to purification of DNA from samples collected at different stages of the oil production chain: ground olives, paste, pomace, unwashed oil, washing water, washed oil. Degradation of DNA occurs mostly in the stages of paste and pomace production. In general, uniformity of bands and profiles of molecular markers was ascertained during all stages from olive to oil.The search for new and improved molecular markers was carried on in parallel on leaf material and monovarietal oils of specific European cultivars, chosen because of their importance in production of PDO and PGI oils; non European cultivars were also included for comparison purposes. Molecular markers which have been assessed for identification of cultivar-diagnostic markers are based on DNA amplification with PCR. Some of them were already in use for phylogenetic studies of olive cultivars and species, whereas others are being developed specifically for the project. Locus-specific markers such as microsatellites are being chosen among published data and newly developed libraries. Multibanded markers, such as RAPD and AFLP, are providing fragments to be cloned and sequenced to design specific sequence-tagged primers. All cloned fragments were verified to be amplifiable also in DNA from oil. SNPs are being developed from protein-coding genes, chosen among those known to be involved in the biosynthetic pathways of specific oil components. Polymorphisms were identified by sequencing and microsequencing. All available polymorphic markers will then be used to develop primers and probes for quantitative RealTime PCR or microarrays.Main metabolites which are characteristic of olive oil have also been purified, linking their concentration and diversity with the region of origin of the oil, especially in PDO and PGI oils: phenols, polyphenols, tocopherols, fatty acids, triacylglycerols, sterols. Metabolites have been extracted from monovarietal olive oils and analysed through HPLC, GC or colorimetric procedures. The most stable components of oil are fatty acids and triacylglycerols. These parameters allow a classification of oils and detection of adulteration from oils of other plant species. With discriminant analysis it is possible to classify samples from monovarietal and PDO oils into different groups. The clustering of groups can give indications on genetic background and geographical origin. This study has been carried out with financial support from the Commission of the European Communities, specific RTD programme "Quality of Life and Management of Living Resources", QLK1-CT-2002-02386, "Traceability of origin and authenticity of olive oil by combined genomic and metabolomic approaches (OLIV-TRACK)". It does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

EVIDENCE OF INTRA-CULTIVAR GENETIC VARIABILITY IN OLIVE CULTIVARS

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130 olive samples, corresponding to 67 different cultivar denominations from several Mediterranean countries, were genotyped at 14 microsatellite loci, yielding 100 genotypes. A total of 135 alleles with a mean number of 9.6 alleles per locus were detected. As different allelic profiles were obtained from putatively synonymous cultivars, several cases of synonymy listed in the FAO database were refuted. In some denominations there were allele differences of up to 60%, indicating the existence of homonyms or mislabeled samples in olive germplasm collections. An allele-sharing phenogram of the analyzed genotypes revealed several cultivars with high levels of intra-varietal polymorphism, as well as cultivar families consisting of closely related cultivars with similar denominations. This work shows that the current designations of olive cultivars insufficiently describe the existing genetic variability

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DEVELOPMENT AND CHARACTERIZATION OF 12 NEW MICROSATELLITES IN OLIVE (Olea europaea L.)

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The genetic diversity existing in the cultivated olive (Olea europaea L.) is enormous. For some time now, scientists have made use of DNA-based markers for a better genetic knowledge of olive. The goal of this report is to announce 12 new SSRs in olive and evaluate their polymorphism within the species. A genomic library was enriched for GA, GT and ACT repeats sequences. Primer pairs were designed for 24 sequences containing SSR. Four of them failed to amplify, eight produced spurious bands or complex bands patterns and 12 succeeded in giving reliable PCR products. Considering these 12 primer pairs, ten of them showed single locus amplification whereas the other two revealed two loci each. The multi-locus nature of these two SSRs and their inheritance fashion were studied in progenies from 'Manzanilla de Sevilla' × 'Arbequina' and 'Picual' × 'Arbequina' crossings. All the expected classes were found in the segregation of the four loci evaluated. Only one locus did not exhibit Mendelian inheritance and the presence of a null allele was confirmed in the paternal genotype of the second cross. A total of 85 alleles were detected for these 12 SSRs when analysing 51 olive cultivars. The number of alleles per locus ranged from 1 to 15. Twenty-five out of the 85 alleles identified were unique. The expected heterozygosity varied between 0 and 0.835.

CULTIVAR IDENTIFICATION AND ELUCIDATION OF GENETIC RELATIONSHIPS WITHIN THE SPECIES Olea europaea L. USING MICROSATELLITES

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The genetic knowledge about the species Olea europaea L. and the evolutionary relationships among the most extensively employed cultivars is limited. For this reason, the purpose of the present report is to test the suitability of 15 available microsatellites for identifying 51 olive cultivars and elucidating the phylogenetic relationships within the species. All pairs of cultivars except one ('Cornicabra'-'Morisca') could be distinguished using only three SSRs, thanks to the high presence of unique alleles (10 out of 38 in those three SSRs and 39 out of 122 when 15 markers were considered) and genotypes (42 out of 62 in those three SSRs and 102 out of 182 for the complete set of primers), but it was necessary to employ an additional one for discriminating all of them. The cultivar with more unique alleles (four) was 'Zaity'. The discrimination power of every SSR was calculated in order to evaluate their efficiency in cultivar identification and set comparisons among them. SSRs with discrimination power value higher than 0.8 resulted especially useful for distinguishing cultivars. The data coming from genotyping the 51 olive cultivars with the 15 SSRs were used for constructing a dendrogram by UPGMA cluster analysis using the Dice similarity coefficient. Cultivars association according to their geographical origin was not observed. In contrast, the Spanish varieties showed certain tendency to group by their origin zones.