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DISCOVERY OF DNA POLYMORPHISMS IN OLIVE *fad7* GENE BY ECOTILLING

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Olive (*Olea europaea* L.) is a subtropical woody species distributed throughout the Mediterranean regions whose remarkable importance is due mainly to the oil production from its drupes. The ancient origin and the ancestral hybridisation between different species of genus *Olea* and between genetically distant populations has originated numerous varieties. Unlike other crops, olive germplasm has not suffered any genetic erosion because a turnover with new genotypes has not occurred and old plants are able to survive for a long time without cultivation. Therefore a large variability has been preserved until now, but it has not been depth studied jet. Knowledge about evolution and genetic relationships within available germplasm are helpful to allow a better choice of parental lines to be used in crosses and to enlarge the genetic basis in olive breeding programs.

A new strategy for SNP detection and characterization in natural populations is represented by EcoTILLING, a variation of TILLING which has been successfully used to examine genetic variation and to genotype several species. In combination with sequencing, EcoTILLING is an high throughput and very cost-effective technology, that allows to simultaneously screen a big number of individuals and to detect natural polymorphisms in coding regions of target genes. Moreover, individuals can be grouped according to their aplotype and distinguished in homozygous and heterozygous; finally, the effect of each identified mutation on protein structure and function can be predicted.

The screening of several web-databases and the ortholog sequences of the *fad7* gene (responsible of the 18:2 to 18:3 fatty acid desaturation) led to the identification of two olive ESTs. The sequence encoding for the fad7chloroplastic isoform was chosen as candidate gene. The genomic sequence and the structure of the gene were obtained by sequencing several overlapping PCR products; moreover, bioinformatic analysis allowed to predict the most suitable region of the gene for EcoTILLING screening.

The application of the EcoTILLING strategy to olive genome was optimized for a subset of accessions, including cultivars and clones of the same cultivar. The *Cel*I-based mutation assay and the Li-COR electrophoresis were chosen as SNP detection system. The cultivar Leccino was used as reference for the constitution of the 2-fold pools. Few polymorphisms were identified between the cultivars, confirming the high level of conservation of this gene. For example, a SNP at 630bp of the analyzed fragment allowed to distinguish the Leccino, Nociara, Toscanina and Frantoio cultivars (aplotype 1) from Cima di Melfi and Ascolana Tenera cultivars (aplotype 2). In the case of Leccino, the analysis among the 5 clones has revealed no genetic variation at the screened locus, showing the uniformity of the Leccino samples.

Additionally, to detect the heterozygosity level in the target gene, single DNA from each cultivar was analysed, supposing that an heterozygous mutation in a diploid genome could be identified by the heteroduplex formation between the wildtype allele and its mutated counterpart.