

DEVELOPING SNP MARKERS FROM CANDIDATE GENES IN OLIVE

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The identification of SNP (Single Nucleotide Polymorphism) markers has assumed a particular relevance in olive genetics for the screening of the germplasm resources, for mapping purposes and for varietal discrimination as well.

The identification of SNPs has been oriented toward specific coding sequences involved in the phenylpropanoid and fatty acid synthesis and in other important pathways.

For the identification of the olive candidate genes degenerated primers, designed on conserved regions of the genes previously identified in other species, were used for PCR amplification of the homologous in olive cDNA, cv. Leccino. After cloning, the Genome Walking technique was adopted and sequences were extended on both sides of the clones, in order to recover the full-length gene. To identify potential SNPs several portions of the genes were directly sequenced on a restricted number of cultivars. Once a polymorphic region was discovered, a systematic direct sequencing of that region on a wider set of cultivars was performed.

On a region of the phenylalanine ammonia-lyase (PAL) gene (714 bp), 3 SNPs and 1 indel were screened.

From the analysis of a 932 bp fragment of the Acyl Carrier Protein (ACP) gene containing a 684 bp intron 5 SNP and 1 indel of 10 bp were identified.

An actin gene has also been identified and the presence of 5 SNPs was retrieved from a 426 bp fragment.

Most of the SNP identified were heterozygous and, as expected, the intron regions have shown the highest variability. For the 3 regions analysed a SNP every 138 bp has been encountered.

The identification of the corresponding aplotypes has been undertaken on a set of 10 cultivars.