Proceedings of the 52nd Italian Society of Agricultural Genetics Annual Congress
Padova, Italy – 14/17 September, 2008
ISBN **978-88-900622-8-5**

Poster Abstract – F.12

DEVELOPMENT OF NEW MOLECULAR MARKERS FOR CULTIVARS IDENTIFICATION IN *OLEA EUROPAEA* L.

INTRIERI M.C., NIEDDU F., GIANNONI A., PARENTI N., LIBRANDI L., BLOISE M., BOGANI P., MULEO R., BUIATTI M.

Dipartimento di Biologia Evoluzionistica "Leo Pardi", Via Romana 17, 50125 Firenze (FI)

Olea europaea, fluorescent-ISSR, TRAP, cp-SSR, trnT-trnD

The *Olea europaea* cultivars identification is particularly complex due to the great number of local accessions, the synonymy and the ambiguous cultivar assignments. On the other hand, unambiguous characterisation is a crucial cue of modern horticulture, with application in breeding programmes, in germplasm collections management and in products traceability. Genetic fingerprinting seems to be the best way for genotyping the specific cultivar and the oil deriving from it. To date the SSRs fingerprint is the most used method to identify the cultivar. In addition to SSRs, the molecular identification should be based on the development other kind of unambiguous markers and, in the light of the genomic progress, the molecular marker should be targeted on functional regions.

In this work 43 cultivar of *O. europaea* belonging to the germoplasm collection of "Istituto Sperimentale per l'Olivicoltura" have been analysed, with the aim to identify simple and reliable PCR based markers useful in cultivars identification. The molecular markers chosen for our analysis are ISSR (inter simple sequence repeats, Zietkiewicz et al., 1994 *Genomics* 20:176-183) and TRAP (targeted region amplified polymorphism, Hu and Vick *Pl. Mol. Biol. Reporter* 21:289–294) techniques and chloroplastic markers. The first method is based on the amplification of genomic DNA by a single primer formed by a microsatellite and a fixed sequence as 3' or 5' anchors. The second, TRAP, uses expressed sequence information and a bioinformatics approach to generate polymorphic markers around targeted candidate gene sequences. In particular we designed primers anchored on genes involved in fatty acid metabolism and expressed during the drupe maturation. To improve the resolution power of these markers we set a protocol for the visualisation of the patterns obtained by PCR amplification by means of automated laser fluorescent sequencer (ABI Prism 3100). Moreover we used chloroplastic PCR primers trnT-trnD to amplify the corresponding intergenic spacer (Demesure et al. 1995, Mol. Ecol. 4:129-31) and a set of 10 primers for SSR of the chloroplastic DNA (Weising and Gardner, 1999, Genome 42-.9-19; Kung and Staub, 2003, TAG 107:757-767). All sequence reactions were made using the AutoRead Sequencing kit. Sequence reaction and the SSR amplicons were run in an automated laser fluorescent sequencer (ABI Prism3100). The intergenic trnT/trnD spacer is able to identify two of the 22 cultivars analysed. Only a point mutation is present among the cultivars. The chloroplastic SSR markers analysed in this work show a sufficient pattern of variation to be able to discriminate some cultivars.

In the second phases of the work we set and verified the repeatability of the fluorescent ISSR and TRAP methods (in fact in general the methods based on random or pseudo-random PCR amplification suffer of a lack of reliability). In our cases both ISSR and TRAP gives good results giving rise to a mean reliability around 95% that is the common standard reported in literature. If

the limits of the protocol set is the small window of fragment dimensions read by the sequencer, on the other hand the speed and the simplicity make the fluorescent ISSR technique a fast and easy method to process a large number of samples in few days. Moreover TRAP based on functionally relevant loci, may became a method useful to perform a wide screening to find possible functionally relevant polymorphisms.