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HIGH-RESOLUTION DNA MELTING CURVE ANALYSIS TO ESTABLISH *PHYA* GENOTYPIC IDENTITY WITH SATURATING DNA DYE IN *OLEA EUROPAEA* L.

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The importance of phytochrome A (PHYA) in plant development has long been recognized. Current studies continue to show a correlation between degree of mutation of *phyA* gene and the regulative role into signal transduction of PHYA. *phyA* has been used in the evolution study of Angiosperms and recently as molecular functional marker for genotyping assay of many cultivated and spontaneous species. Therefore, it is a good candidate as molecular marker in biodiversity analysis, germplasm collections typing and products traceability. Genotyping *O. europaea* is particularly complex due to the broad number of cultivars and the analysis is complicated by the presence of synonymy and ambiguous cultivar assignments. The new challenge in plant genotyping is SNPs assay, which is robust and specific, although the identification of single mutations and the analysis are laborious and expensive.

In this study, sequence portion of *O. europaea phyA* gene has been obtained by PCR amplifications using degenerate primers, designed on an alignment of all the genes present in GenBank. The obtained PCR product of cv. Canino was cloned and sequenced (Accession: AY924378). All subsequent PCR amplifications were performed on 14 *O. europaea* cultivars from different latitude (southern to central region of Italy) and habit, using specific primers that amplified a 307 bp fragment. The DNA templates were used at 50 ng under saturating LCGreen™I fluorescent DNA dye conditions and the reactions were performed with LightCycler (Roche). Melting analysis was performed either on the LightCycler immediately after cycling and on high-resolution melting instrument (HR-1), subsequently. Using LigtCycler and HR-1 softwares we performed continuous acquisition of fluorescence until 89 °C. Normalized and derivate melting curve were calculated by software.

Shape of melting curve and value of Tm were able to distinguish and separate every amplicon of each cultivar. Using the values of Tm we were able to grouped in three main cluster all the genotype. The first cluster identified the most part of cvs from Sicily and Calabria, plus Bardhe di Tirana, and Nociara, which is from the southern part of Apulia (Taranto, Brindisi). The second cluster grouped together the cultivars belonging to the Central region of Italy and those from the region located at the North of Pollino mountains. The third cluster included only *O. europaea var. sylvestris.* Variation in melting curve shape were also present in some of the cultivars indicating that an allelic condition of the gene is present. To validate the obtained results with this technique the *phyA* portion has been sequenced in all cultivars, in order to uncertain if the same haplotype belongs to the same group have high value of homology.

In this work we show the possibility to use this technology to identify probably mutations in unknown sequences as first step of SNPs recognition, through the high melting curve shape and Tm value. Moreover, this technology is also able to identify heterozygotes by a change in melting curve shape.

We present for the time results related with the application of this technology to plant genomic analysis. Finally, now it is available a robust and specific assay, easy to apply and reliable for identification of polymorphisms among genes and cultivars both as first screening of unknown sequences and for SNPs analyses of large number of genotypes..