GENETIC ANALYSIS USING DNA MOLECULAR MARKERS OF THE ‘MALTI’ OLIVE VARIETY FOUND ON THE MALTESE ISLANDS

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Introduction

There are over 1,200 known olive cultivars, most of which require different environmental and geographical conditions for optimal growth and often produce olives of varying quality. At present the main criteria used to distinguish between olive cultivars include phenotypic characteristics, isozyme biochemical markers and compositional markers. DNA based compositional markers may be considered the most effective and were therefore selected for this study. The main aim of this study was to investigate whether molecular markers could be used to identify the ‘Malti’ cultivar. Objectives to achieve this were:

I. To select and optimise an efficient procedure for DNA extraction from these olive trees.
II. To use random amplified polymorphic DNA (RAPD) to try to derive markers. DNA based compositional markers may be considered the most effective and were therefore selected for this study. The main aim of this study was to investigate whether molecular markers could be used to identify the ‘Malti’ cultivar. Objectives to achieve this were:

Methodology

Five different extraction methods were tested, three of which involved modifications of the CTAB method proposed by Doyle and Doyle (1987), while two extraction methods involved the use of commercially available DNA extraction kits (Vivantis GF-1 Plant DNA Extraction Kit and AccuPrep GMO DNA Extraction Kit). Once the extraction procedure was established DNA was extracted from ancient ‘Malti’ olive trees situated in Lija, Mellieha and Zebbug. Olive trees of the ‘Bidni’ and ‘Cetrala’ variety were also included. The nine RAPD primers selected for genetic analysis included OP-A18, OPC-19, OPA-17, OPA-07, OP-B-15, OP-16, OPAN-15 and OPAG-13. The PCR banding patterns produced for each sample were analysed for similarity using Jaccard’s coefficient (1908), Nei and Li’s coefficient (1979) and the simple matching coefficient. Nei’s genetic distance coupled with UPGMA cluster analysis was used to construct a dendrogram.

Results

![Figure 1A - 0.7% agarose gel of Lija 2 and Lija 4 genomic DNA extracted using CTAB 2 - lanes 1 and 2, Accuprep GMO kit - lanes 3 and 4, Vivantis GF-1 DNA extraction kit - lanes 5 and 6, CTAB 3 - lanes 7 and 8, Lane 9: Lambda DNA/EcoRI + Hind III Marker](image1)

![Figure 1B - 0.7% agarose gel of genomic DNA for all six olive variants extracted using CTAB 1. Lane 1 - Bidni, Lane 2 - Mellieha, Lane 3 - Lija 2, Lane 4 - Sicily, Lane 5 - Wied Qirda, Lane 6 - Lija 4, Lane 7: Lambda DNA/EcoRI + Hind III Marker](image2)

![Figure 2 - Loci Specific to Particular olive samples and groups of samples generated using RAPD primers](image3)

![Figure 3 - Dendrograms based on (a) Nei's genetics distance and (b) Jaccard's coefficient between the different olive samples.](image4)

Conclusions

CTAB method 1 which included slight modifications with respect to the procedure originally proposed by Doyle and Doyle (1987) was found to be the most effective DNA extraction method, yielding a mean DNA concentration of 199.34 ± 41.37 ng/µL, A260/280 nm of 2.00 ± 0.37 and A260/230 nm of 1.44 ± 0.39. Genetic analysis based on RAPD primers suggested that the ‘Malti’ olive trees show a high degree of genetic variability. The results may also indicate that the ‘Bidni’ variety is indigenous to the Maltese Islands, however further analysis is required. A number of loci were also found to be specific to particular olive trees. This preliminary identification of olive trees may be useful to the local olive oil production industry, and could eventually lead to PDO certification of some Maltese produced olive oils.

References


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