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 molecular markers for the variety.

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, Mellieħa and Żebbuġ. Olive trees of the 'Bidni' and 'Cetrala' variety were also included. The nine RAPD primers selected for genetic analysis included OPI-18, OPC-19, OPA-17, OPA-07, OPBC-05, OPB-18, OPA-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

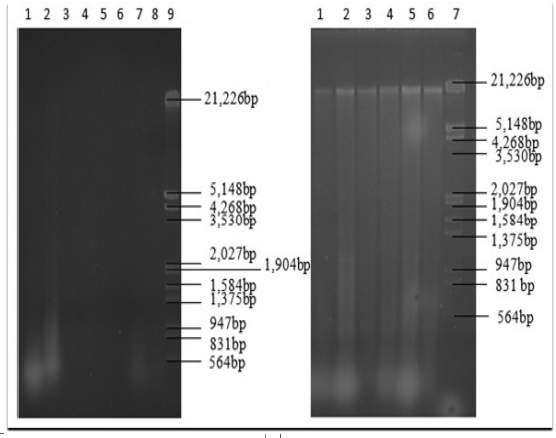
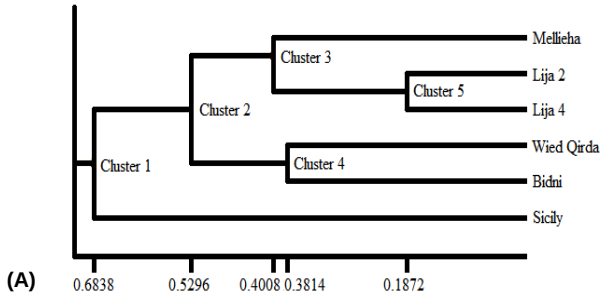


Figure 1A – 0.7% agarose gel of Lija 2 and Lija 4 genomic DNA extracted using:
- •CTAB 2 - lanes 1 and 2
- •AccuPrep® GMO kit - lanes 3 and 4
- •Vivantis GF-1 DNA extraction kit - lanes 5 and 6
- •CTAB 3 - lanes 7 and 8
- •Lane 9: Lambda DNA/EcoRI + Hind III Marker

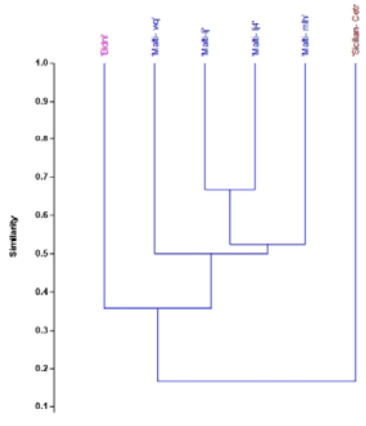
Figure 1B – 0.7% agarose gel of genomic DNA for all six olive variants extracted using CTAB 1.
Lane 1 – Bidni
Lane 2 – Mellieħa
Lane 3 – Lija 2
Lane 4 – Sicily
Lane 5 – Wied Qirda
Lane 6 – Lija 4
Lane 7: Lambda DNA/EcoRI + Hind III Marker

Discriminating loci	Olive Variety(ies)
OPA-17 (at 630 bp) and OPAG-13 (at 250 bp)	Common to samples Lija 2, Lija 4 and Mellieħa only
OPI-18 (at 650 bp and 500 bp), OPC-19 (at 460 bp and 300 bp) and OPA-17 (at 800 bp)	Common to all the Maltese varieties (Mellieħa, Lija 2, Lija 4, Wied Qirda and Bidni) but absent in Sicily
OPA-05 (at 450 bp) and OPAG-13 (at 150 bp)	Present in Bidni variety only
OPA-17 (at 422 bp) and OPA-07 (at 350 bp)	Present in Wied Qirda sample only
OPA-07 (at 900 bp), OPB-18 (at 800 bp), OPA-16 (at 800 bp) and OPAN-15 (at 150 bp)	Present in Sicilian (Cetrala) sample only

Figure 2 – Loci Specific to Particular olive samples and groups of samples generated using RAPD primers



(A)



(B)

Figure 3 – Dendrograms based on (a) Nei's genetics distance and (b) Jaccard's coefficient between the different olive samples,

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

[1] Doyle, J. A.; Doyle, J. L. A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochemical Bulletin* **1987**, *19*, 11–15.

Acknowledgements

The authors would like to thank Mr. Sam Cremona and Mrs. Matty Cremona for providing samples and invaluable information. Special thanks also goes to Prof. Gary Hunter and Dr. Therese Hunter for use of the research laboratories.