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Establishment of a reliable protocol for gDNA extraction from olive oil

OLIVE GROVES
OLIVE OIL

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Por3O · QREN Quadro de Referência Estratégico Nacional Portugal 2007-2013 · União Europeia · Fundo Europeu de Desenvolvimento Regional · COMPETE Programa Operacional Factores de Competitividade · FCT Fundação para a Ciência e a Tecnologia · Ministério da Educação e Ciência · República Portuguesa

INTRODUCTION

With no possibility to reach in quantity the production of countries with large areas of olive orchards, as a small country, Portugal needs to define a strategy for valuing the high quality and specificities of its olive oil. No doubt the focus must be on the valorization of the Portuguese cultivars, the key factor in determining the singularity of the produced olive oils. Fraud detection, as the use of non-Portuguese varieties, is the main aim of varietal and DOP olive oil producers. In this sense, it is mandatory to have tools allowing the control of the varietie(s) giving rise to the olive oil, both in quality and in quantity.

One objective of the project *Por3O* is the establishment of a molecular tool that identifies the varieties used to produce a given olive oil. Ideally, this tool could be further proposed for screening for frauds and to support olive oil certification. However, as PCR-based tool, it requires the availability of genomic DNA (gDNA) with quality enough to be used on fragment amplification. Here we will describe a robust and efficient gDNA extraction protocol, which allow its further use for Single-Sequence Repeats (SSRs) markers amplification. Several DNA commercial kits will be here compared on its capacity to extract gDNA from a commercial blend olive oil and its applicability on SSRs amplification.

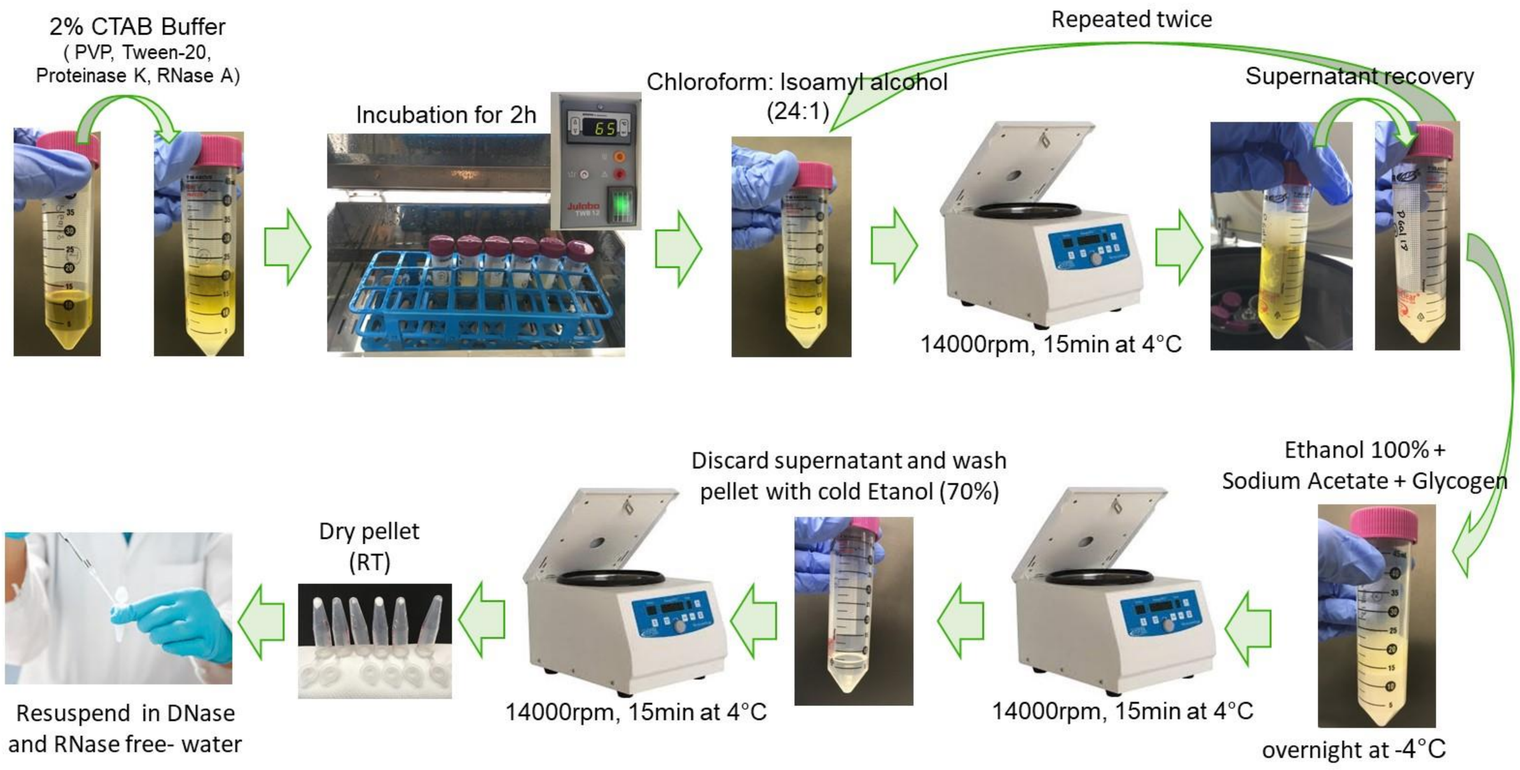
MATERIALS AND METHODS

Establishment

Ten different commercial kits (more information in Table below) were tested on its capacity to isolate genomic DNA (gDNA) from a commercial blend olive oil and they were compared with an in-house method based on CTAB-based protocol previously published [1] including some modifications (see procedure on the right).
The different methods were compared in terms of starting volume of oil sample required for extraction, average gDNA concentration, total gDNA extraction yield (see results in Table below) and efficiency in Single-Sequence Repeats (SSRs) markers amplification (SsrOeUA-DCA4, SsrOeUA-DCA9 and SsrOeUA-DCA11).

Validation

For procedure validation gDNA was isolated following the the in-house protocol, from two olive oils (Abencor system) per cultivar and a commercial monovarietal olive oil. Three cultivars were considered: 'Cobrançosa', 'Galega vulgar' and 'Arbequina'. In total, gDNA was isolated from 9 olive oils.
Same SSRs tested for gDNA extracted with kits were here amplified. gDNA isolated from leaves of the same cultivars was used as control.

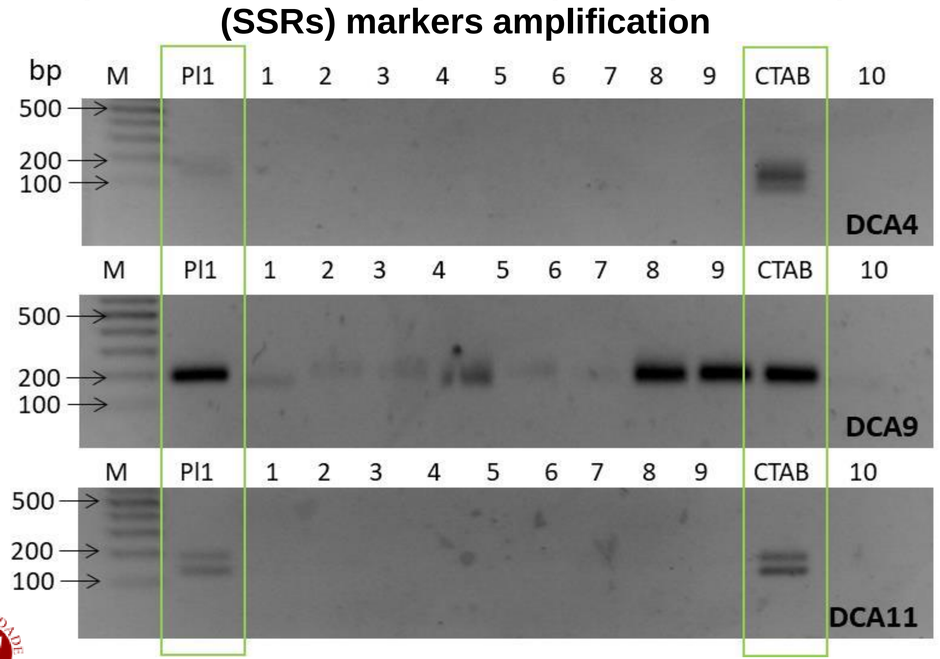


RESULTS AND DISCUSSION

Comparison of different methods for gDNA isolation

	Method	Starting vol. (µL)	[gDNA] (ng/µL)	Eluted (µL)	Yield (ng)	Purity ($A_{260/280}$)	Purity ($A_{260/230}$)
1	DNeasy Plant Mini Kit (QIAGEN)	200	2	250	575	2.57	0.16
2	NucleoSpin Plant II kit (MN)	200	28	100	2800	1.44	0.50
3	GeneJet Plant Genomic DNA Purification Mini kit (Thermo)	200	6	100	630	0.88	0.48
4	GeneJet Genomics DNA Purification kit (Fermentas)	200	25	250	6100	1.22	0.38
5	innuPREP Plant DNA kit (AG)	200	13	80	1024	0.79	0.08
6	Biomics DNA kit (Zymo)	200	17	40	672	0.75	0.32
7	Quick DNA (Zymo)	200	22	100	2150	1.08	0.40
8	LEV (Promega)	200	24	30	708	0.88	0.31
9	SEV (Promega)	50	26	30	768	0.86	0.29
10	QIAmp PowerFecal DNA kit (QIAGEN)	200	9	30	270	1.14	0.57
CTAB	CTAB	1000	24	20	478	1.08	0.13

Comparison on efficiency in Simple-Sequence Repeats (SSRs) markers amplification

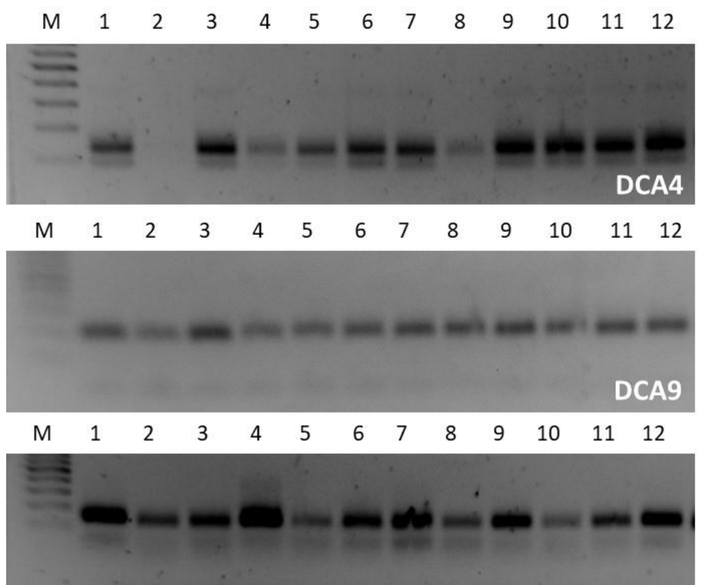


- ✓DNA commercial kits have the advantage of higher reproducibility, greatly removing technician expertise biases;
- ✓It requires small amounts of sample volume.

However…

gDNA isolated with commercial kit was not efficient in SSRs markers amplification

The in-house method, even though requiring more time for gDNA isolation and more volume to proceed with the isolation protocol, resulted in a much higher efficiency in SSRS markers amplification.



Legend:
1 - Plant 'Cobrançosa'
2 - Cob1 (Abencor)
3 - Cob5 (Abencor)
4 - 'Cobrançosa' commercial olive oil
5 - Plant 'Galega vulgar'
6 - Gal18 (Abencor)
7 - Gal22 (Abencor)
8 - 'Galega' commercial olive oil
9 - Plant 'Arbequina'
10 - Arb4 (Abencor)
11- Arb9 (Abencor)
12 - 'Arbequina' commercial olive oil

REFERENCES

[1]- Raieta K., Muccillo L. and V. Colantuoni, A novel reliable method of DNA extraction from olive oil suitable for molecular traceability. Food Chem., 2015. 172: 596-602.

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