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# DNA extraction from Clusterbean (*Cyamopsis tetragonoloba*)

# Above-ground biomass estimation of *Opuntia elata* an invasive species in Portugal

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## Abstract

The objectives of this work were: i) to test a commercial kit (DNeasy® Plant Mini Kit) in the DNA extraction from guar or clusterbean leaves (*Cyamopsis tetragonoloba*); ii) the determination of biometric parameters in *Opunta elata* cladodes with the aim to develop biometric equations that allow the above-ground biomass estimation of *Opuntia elata*, an invasive species in Portugal. The first part of the work presents general information about clusterbean as well as the methodology and materials needed for DNA extraction from guar plant. It was therefore necessary to both define and discuss the issue of DNA quantification and purity estimation. DNA extractions were conducted on four selected lines (G67, G86, G91 and G96) and one cultivar (RGC 936) of guar. The DNA yield and purity estimation were determined both by gel agarose electrophoresis and spectrophotometry. We can conclude that the tested kit can be used in DNA extraction from guar leaves in future studies. With the exception of sample 91, the extraction method produces DNA samples with acceptable purity ratios and they can be used in downstream applications such as PCR analysis. The second part of the work contains information about the botany and morphology of *Opuntia elata* and data from biometric parameters quantified in *Opuntia elata* cladodes. We developed linear models using biometric data from the cladodes to predict the fresh weight. The last part of the thesis contains DNA extraction protocols and information on agarose gel electrophoresis. Conducted research was presented in both descriptive and tabular form, as well as in pictures.



## Key words

DNA extraction, clusterbean, agarose gel electrophoresis, *Opuntia elata*.

## Resumo

Os objetivos deste trabalho foram os seguintes: i) testar um kit comercial (DNeasy® Plant Mini Kit) na extração de DNA da folha de guar (*Cyamopsis tetragonoloba*); ii) fazer a determinação de parâmetros biométricos em cladódios da espécie invasiva *Opunta elata,* com o objetivo de desenvolver equações por regressão linear, que permitam estimar a produção de biomassa. A primeira parte do presente relatório apresenta informação geral acerca do guar bem como metodologias utilizadas na extração de DNA. São abordadas algumas técnicas usadas na quantificação e análise de pureza do DNA. As extrações de DNA foram realizadas em quatro linhas de guar previamente selecionadas (G67, G86, G91 e G96) e na cultivar RGC 936. O rendimento em DNA e a pureza foram determinados por eletroforese em gel de agarose e por espectrofotometria. Podemos concluir que o kit de DNA testado pode ser utilizado em trabalhos futuros. Com exceção da amostra 91, o método de extração utilizado permite obter DNA com grau de pureza aceitável e pode ser utilizado em aplicações a jusante como a análise PCR. A segunda parte do trabalho contém informação botânica acerca da espécie invasiva *Opuntia elata* e dados biométricos quantificados em cladódios. Com os dados biométricos dos cladódios foi desenvolvida, por regressão linear, uma equação que permite quantificar, por métodos não destrutivos, a produção de massa verde. Na parte final do trabalho são apresentados os protocolos utilizados na extração de DNA e na eletroforese horizontal em gel de agarose.



## Palavras-chave

Extração de DNA, guar, eletroforese em gel de agarose, *Opuntia elata*.

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# Abbreviations

A260 - Absorbance at 260 nm

A280- Absorbance at 280 nm

AE – Tris, EDTA elution buffer

AFLP - Amplified fragment length polymorphism

ANOVA- Analysis of variance

AP1- DNA extraction buffer

AW1, AW2 - DNA extraction buffers

COVID 19 – Coronavirus disease 2019

CV (%) - coefficient of variation

ddPCR – Droplet digital PCR

DM - Dry matter

DNA -Deoxyribonucleic acid

dsDNA - Doubled stranded DNA

DW- Dry weight

EDTA - Ethylenediaminetetraacetic acid

ESACB - Escola Superior Agraria de Castelo Branco

EtBr - Ethidium bromide

FW - Fresh weight

G – Gram

L – Cladode length

Max - Maximum

Min - Minimum

P3 – DNA extraction buffer

PCR - Polymerase chain reaction

qPCR - Real time polymerase chain reaction

RNA - Ribonucleic acid

RNase A - bovine pancreatic ribonuclease

SD - Standard deviation

SDS - Sodium dodecyl sulfate

T – Cladode thickness

TAE - Tris, Acetic acid, EDTA buffer

TE - Tris-HCl, $Na_2EDTA$ Buffer

TGW - Thousand grain weight

UV – Ultraviolet

W – Cladode width