DNA Isolation from Desiccated Leaf Material for Plum Tree (*Prunus domestica* L.) Molecular Analysis

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Abstract

Conservation of biodiversity is very important, especially in species such as plum tree. One of the key aspects is the establishment of appropriate genetic markers that could be used to identify provenances and also for breeding purposes. In this context, molecular characterization using SSR (short sequence repeats) markers is very useful and was chosen for our study. This article describes the assessment of a CTAB-based DNA extraction protocol for isolating and purifying DNA from the plum cultivars selected for molecular characterisation. The quality of DNA extracts was assessed by spectrophotometric measurement and their suitability for molecular analysis was evaluated using a SSR marker system. We concluded that the DNA extraction protocol is suitable for obtaining plum DNA that can be used for molecular analysis using SSR markers.

Keywords: *plum tree, $CaCl_2$, DNA isolation, CTAB, marker, SSR.*

INTRODUCTION

Conservation of biological diversity is important within each species and especially for economically relevant ones, such as plum tree (*Prunus domestica* L.), which accounts for more than one third of Romania's fruit trees acreage. In this context, one important aspect is the phenotypical and molecular characterisation of local populations and old, traditional cultivars. Identified markers should firstly constitute an effective means for identification of each provenance and they could also prove very useful in any breeding applications.

AIM AND OBJECTIVES

The aim of our project is to create a database comprising phenotypic and SSR-derived (short sequence repeats) molecular characteristics of Romanian plum tree cultivars. One of the key objectives is the proper isolation of DNA from all plum tree cultivars in order for the SSR analysis to be performed. The establishment and optimization of this procedure for our study is described here.

MATERIALS AND METHODS

The 70 plum tree cultivars selected for SSR analysis (Table 1) are part of the SDPV Vâlcea collection (Râmnicu Vâlcea, Romania). Fresh leaves obtained from each cultivar were incubated for approximately two weeks at 4 °C, with $CaCl_2$, for desiccation. A quantity of 0.5 g of dried material was then grinded using a TissueLyser II mixer mill (Qiagen) and the obtained powder was used directly for DNA extraction.

DNA extraction was performed using the CTAB-based method published by Lodhi *et al.* (1994) and improved by Pop *et al.* (2003). Our modification to the protocol was skipping the grinding of samples in liquid nitrogen, which were prepared as previously described.

The purity and concentration of DNA extracts were determined spectrophotometricaly, using the NanoDrop1000 instrument (NanoDrop Technologies).

The quality of extracted DNA was further tested by PCR, employing a SSR marker system in which amplification reactions were carried out

Tab. 1. Plum tree cultivars selected for molecular analysis from the collection held at SCDP Vălcea

Andreea	CT 163	Lăudatu	Presenta	Sâmbata 1
Alutus	Corval	Minerva	Păscoaia 4	Tita
Alina	Diana	Mici de Stoicești	Plumis	Tuleu gras
Aurii de Bistrița	de Botești	Molive	Prun de Apold	Tuleu timpuriu
Balada 4	Dumbrăvești	Mirobolan Dwarf	Rival	Tuleu de Sinești
Bărăgan 17	P. Dumbrăvești	Miroval	Renclod de Caransebeș	Topval
Boambe de Leordeni	Flora	Oltval	Roșior văratec	Troianu 6
Buburuz	Gras ameliorat	Oltenal	Record	Troianu 9
Carpatin	Gogoșele Otășău 3	Oteșani 1	Roz de Densus	Troianu 10
Centenar	Gogoșele Otășău 5	Oteșani 8	Roze tari	Troianu 11
Călugărești T2	Gogoșele Otășău 11	K5	Romandreea	Vâlcean
Călugărești T1	Grase de Pește ana	Pescăruș	Sâmbata 2	Vânăt românesc
Corcoduș de Sâmburești	Gogoșele de Călimănești	Porumbele	Sâmbata 4	Voinești B
CS 2A P9	Goldane negre	Pinval	Scolduș	Vânăt românesc T3

as described by Struss *et al.* (2003), with some modifications. Reaction mixtures (total volume of 12 µL) consisted of 20 ng template DNA, 250 nM of each primer (Generi BioTech), 1.5 mM of $MgCl_2$, 200 µM of dNTPs and 1 U *Taq* polymerase (Promega). DNA amplification was carried out in a 96 well Gradient Palm-Cycler (Corbett Research). Depending on each primer pair, such reactions are run with a touchdown protocol of 30-35 cycles (denaturation at 94ºC for 1 min, annealing at 55-65ºC for 1 min, extension at 72ºC for 1 min), followed by a single extension at 72ºC for 7 min. Two PCR primer pairs were used: UCDCH-13 and UDP 96-001.

The outcome of the PCR amplification was assessed by electrophoresis on a 1.4% agarose gel (Sigma-Aldrich) in 1X TAE, at 0.29 V/cm^2 for 2 hours. The molecular marker used was 100 bp DNA Step Ladder (Promega). Gels were visualized on a UVP Biospectrum AC Imaging System (UVP BioImaging Systems) after 0.5 µg/µl EtBr staining for 20 min.

RESULTS AND DISCUSSION

Using $CaCl_2$ as a desiccating agent was a very efficient approach in terms of costs and handling and, in combination with the incubation temperature of 4 °C, it virtually removed the possibility of microorganism activity. Furthermore, this type of leaf samples is well suited for mixer mill grinding.

The concentration of extracted DNA varied between approximately 220 and 3850 ng/µL, while the 260/280 ratio was between 1.8 and 2.2 for most of the samples. Only four samples had less than 500 ng/µL and almost half exceeded 1500 ng/µL.

All DNA extracts performed well in the PCR experiment using the SSR marker system. Amplicons of appropriate length were identified in each sample analysed using the previously described protocol.

CONCLUSION

We consider that the DNA preparation procedure tested here is very effective in providing extracts well suited for SSR analysis.

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