THE DETERMINATION OF QUANTITY AND PURITY DNA TO SOME AUTOHTONOUS GRAPEVINE VARIETIES

DETERMINAREA CANTITĂȚII ŞI PURITĂȚII ADN LA UNELE SOIURI AUTOHTONE DE VIȚĂ DE VIE

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Abstract. *It was analised the dezoxiribonucleic acid (DNA) to 12 autohtonous grapevine varieties. Total DNA was extrated from young leaves using a modified version from Pop Rodica si colab., 2003 of the protocol publised by Lodhi et al., in 1994. After DNA extraction, the samples was quantified with BioPhotometer Eppendorf help's, was analised the optic density and absorbance ratio at 260 nm and 280 nm. Under raport base betwen these two wave lenghts was established the quantity and purity of DNA at study cultivars.*

Key words: DNA, autochtonous grapevine, vine, extraction, leaves

Rezumat. *A fost analizat acidul dezoxiribonucleic (ADN) la 12 soiuri de vita de vie autohtone. Extractia acidului s-a facut din frunze tinere , utilizandu-se versiunea modificata de Pop Rodica si colab., 2003,a protocolului publicat de Lodhi et. al., 1994. Dupa extractia acidului dezoxiribonucleic, probele au fost cuantificate cu ajutorul aparatului BioPhotometer Eppendorf, analizandu-se densitatea optica si absorbtia la lungimile de unda 260 nm si 280 nm. Pe baza raportului intre cele doua lungimi de unda s-a stabilit cantitatea si puritatea acidului dezoxiribonucleic la soiurile studiate.*

Cuvinte cheie: ADN, soiuri autohtone, viță de vie, extracție, frunze

INTRODUCTION

Studies ampelographic helps to compare morphological characteristics and the characteristics of technological varieties of grapes, to avoid confusion between close varieties and eliminate many synonyms. In time, the methodology used to describe varieties was improved continuously, the extent of accumulation of new knowledge and once with developments in molecular biology. In the current description of the botanical varieties based on morphological criteria is incomplete, being necessary to establish criteria that genetic composition genoms in the varieties of grapes. Along with the emergence of molecular markers, genome investigation was conducted at isoenzyms with protein and DNA-level methods by RAPD, RFLP, AFLP and SSR.In the case of vines, the isolation of DNA and then using it to obtain PCR products is very difficult due to abundance polyphenols, polysaccharides, the presence of RNA and other secondary products (Rout G.R. şi colab., 2002).

MATERIAL AND METHOD

The biological material was represented by young leaves harvested from 12 varieties of grapes are in local ampelographic Collection of the Faculty of Horticulture in Iasi, as follows: Batuta neagra, Busuioaca de Bohotin, Coarna alba, Coarna neagra, Feteasca alba, Feteasca regala, Feteasca neagra, Furmint, Galbena de Odobesti, Grasa de Cotnari, Tamaioasa Romaneasca and Zghihara de Husi.

Technology categories:

1. Varieties for table grapes with late maturation: Coarna alba, Coarna neagra;
2. Varieties for white table wine: Galbena de Odobesti, Zghihara de Husi;
3. Varieties for white wine quality: Grasa de Cotnari, Feteasca alba, Feteasca regala, Furmint;
4. Aromatic varieties for wine: Tamaioasa Romaneasca, Busuioaca de Bohotin;
5. Varieties of red wine for dinner: Batuta neagra;
6. Red wine varieties for quality: Feteasca neagra.

Extraction protocol used is described by Lodhi et al., (1994) and modified (R. Pop et al. (2003). The methods for isolation of DNA and have the basic criteria purity, integrity and quantity of DNA obtained. To obtain the DNA extract were the following stages:

Table 1

Solutions needed for DNA isolation

1.	2 x CTAB 2% - 100 ml	10.	5 M NaCl
2.	2 % CTAB	11.	Buffer TE 100 ml
3.	100 mM Tris HCl	12.	10 mM Tris HCl pH 8
4.	20 mM EDTA	13.	1 mM EDTA phH 8
5.	1,4 M NaCl	14.	Cloroform : alcool izoamilic 24 : 1 - 100 ml
6.	2% PVP	15.	Alcool etilic 95%
7.	10 mM acid ascorbic	16.	Alcool etilic 80 %
8.	4 mM DIECA	17.	RNA enzime A 1 mg/1 ml
9.	Buffer for precipitation - 100 ml		

Is 0.5 g grind biological material in liquid nitrogen to obtain an important powder. Is important by grind not obtain a very fine powder. Add 700 µL of extraction buffer. The PvP, DIECI and ascorbic acid are added to the extraction buffer only when it is used. Transfer about 100 mg of powder and mix thoroughly by reversing tubes Eppendorf. In next incubation tubes for 25 minutes at 65^{0}C and then leave to cool to room temperature. Add 700 µL mixture of chloroform and izoamilic alcohol (24:1) and next well by reversing the tube 20-25 times to obtain an emulsions. It centrifugate for 15 minutes at 11000 r/min at room temperature. Transfer the aqueous phase and then into a new Eppendorf tube.For better extraction, and ensuring a higher purity of the solution of DNA repeat steps 5-6-7. Add 0.5 volume aqueous solution of 5 M NaCl and mix good. In continue to add two volumes of ethyl alcohol 95% cold (-20^{0}C) and keep the tubes in a refrigerator for 15-30 minutes (4-6^{0}C). The solution can be kept in a refrigerator an hour or more if necessary.It centrifugate 3 minutes at 3000 rot/min and then increase speed to 11,000 r/min for 5 minutes at room temperature. This difference helps centrifuge sedimentation of DNA in the centrifuge tube. The supernatant remove and add 700 µL ethyl alcohol 80% cold (0-4^{0}C) for washing the pellets. Centrifugate is 5 minutes at 10000 r/min. Remove the supernatant. Wait until the complete evaporation of alcohol - 20-30 minutes. Pellet is the solution in hydration TE - 50 µL/tube and treated with 1 µL RNA-enzime/100 µL DNA solution and incubation at 37^{0}C for 15 minutes. DNA extract can maintain long-term -70^{0}C or -20^{0}C for shorter periods of time.

RESULTS AND DISCUSSIONS

If this protocol extraction of DNA, the modification consisted in adding the extraction buffer of the following substances: 2% (PvP), 10 mM ascorbic acid and 4 mM dietilditiocarbamic acid (DIECA).

These substances were added before use diluted solutions of DNA. Standard protocol provided by Lodhi et al. (1994) contained in buffer extraction PvP only 2% and 0.2% beta mercaptoetanol. After DNA extraction, samples were quantified using spectofotometric the BioPhotometer Eppendorf.

Spectofotometric method is based on the fact that most organic substances have a rate of absorption feature in the UV radiation: 260 nm for nucleic acids, 280 nm for protein and 230 nm for different contaminants.

Were measured absorbants (optical density) at 260 nm and 280 nm, making the ratio between the two readings A260/A280. DNA is considered sufficiently pure when the ratio of the two acquiring the A260/A280 is between 1.7 and 2.0. Values below 1.7 indicate impurification of protein, and the highest 2.0 impurification with other contaminants.(Fig.1and 2.)

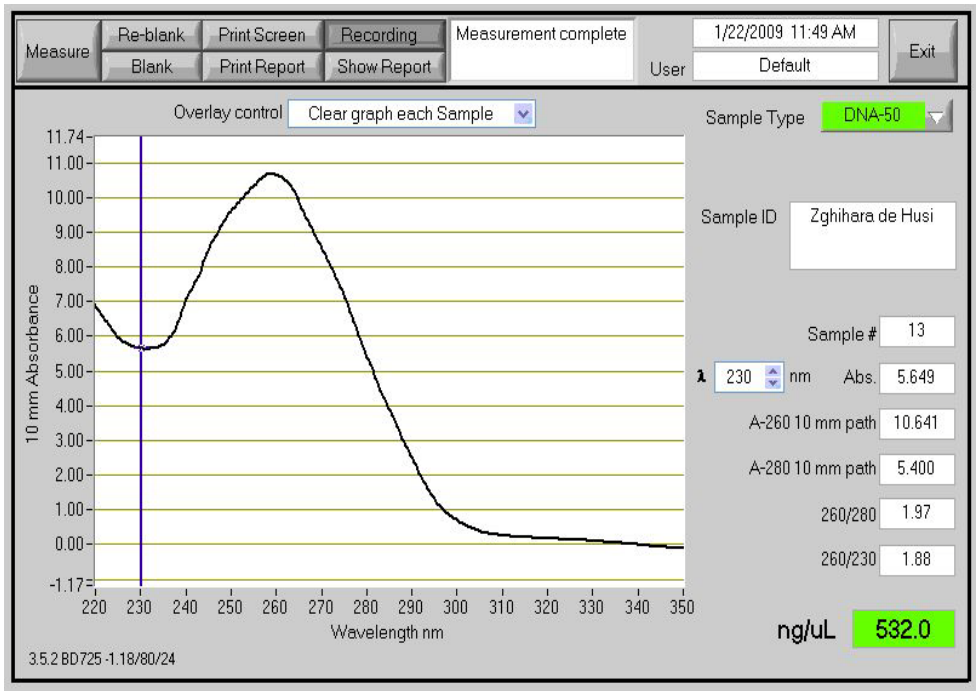

Fig.1. The measure of absorbance of DNA to cultivar Zghihara de Husi

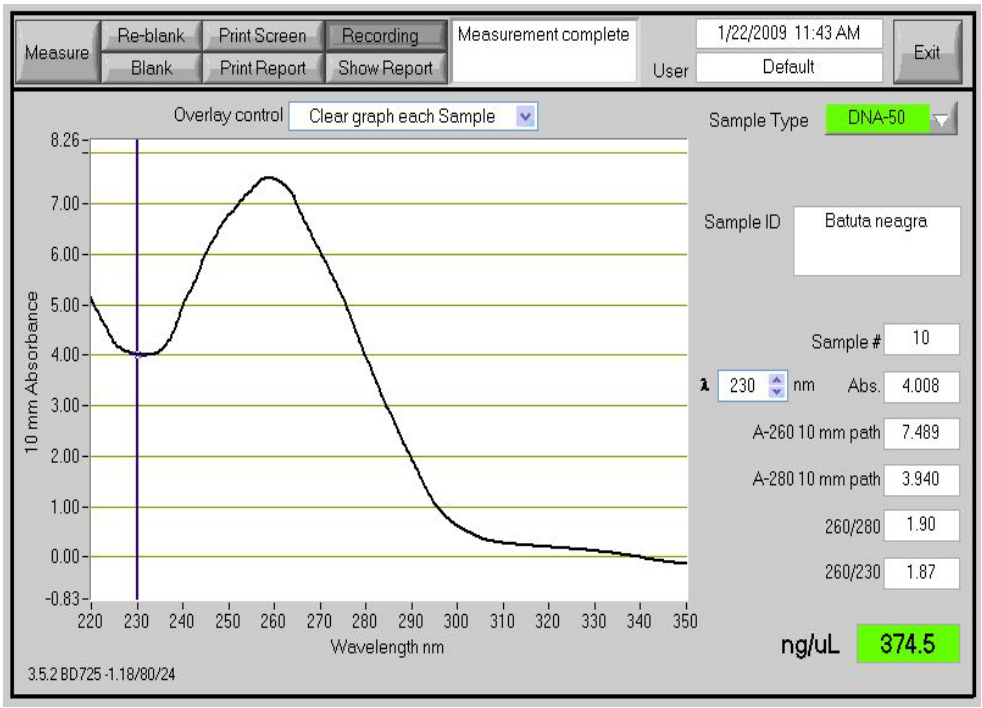

Fig. 2. The measure of absorbance of DNA to cultivar Batuta neagra

The device plays BioPhotometer Eppendorf, both the DNA and the amount of DNA expressed in ng / µL or mg / mL. In the case of the 12 varieties investigated, DNA extracts were differences, both in terms of DNA purity and quantity (tab. 2).

Table 2

The quantity and purity of DNA obtained following extraction

Nr. crt.	Variety	quantity ng / µL	Purity (A260/A280)
1.	Feteasca regala	138,4	1,54
2.	Feteasca alba	169,7	1,72
3.	Feteasca neagra	255,7	1,62
4.	Galbena de Odobesti	136,1	1,87
5.	Zghihara de Husi	532,0	1,97
6.	Batuta neagra	374,5	1,90
7.	Tamaioasa romaneasca	318,2	1,56
8.	Busuioaca de Bohotin	110,1	1,94
9.	Coarna alba	964,9	2,11
10.	Coarna neagra	568,2	1,57
11.	Furmint	438,7	1,66
12.	Grasa de Cotnari	78,6	1,49

A favorable effect on the purity of DNA and had to use the solution of sodium chloride and 5 M absolute alcohol, which increase the solubility polizaharidelor preventing precipitation polysaccharides and a concomitant DNA. Lodhi et al. (1994) shows that addition of sodium chloride in high concentration has been increased in the removal polysaccharides *Vitis species* (Rout et al., 2002). Adding acid and ascorbic acid dietilditiocarbamic (DIECA) proved to be beneficial, the purity of DNA having high values of 1,7-1,9, most varieties investigated. Extraction of DNA by this method led to obtain colorless solution of DNA in most samples analyzed. In figure no. 3 is presented the line and the regression equation and coefficient of correlation between the amount of DNA and its purity determined from the data x harvest time interaction extraction method. In the graph is observed that the relationship between quantity and purity is linear, the individual data group is quite closely around the right of recourse. Correlation between two variables is direct and positive, increasing the quantity of DNA, leading to an increase in its purity.R^2 = 0243 Value ** indicates a significant dependence of the purity of the extracted DNA.

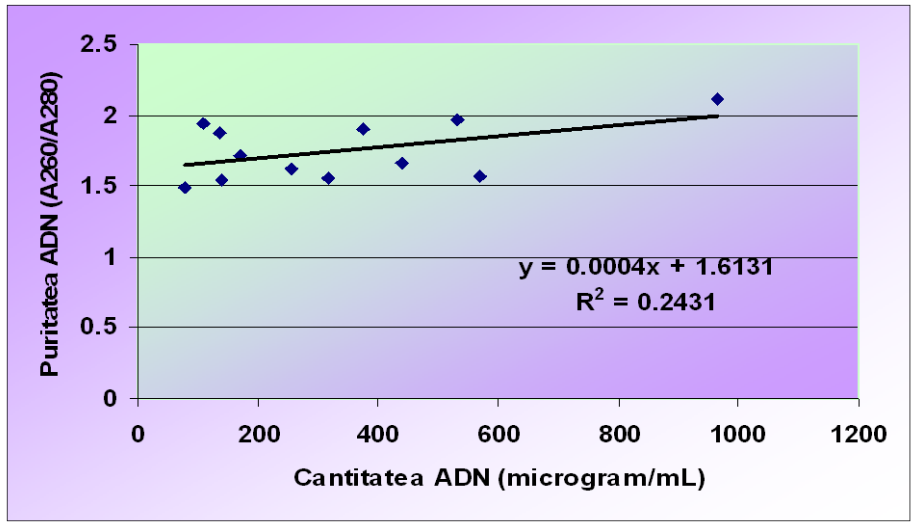

Fig. 3. Right regression equation and the purity of DNA depending on the quantity

CONCLUSIONS

1. Using molecular biology techniques (RAPD, AFLP, SSR) in the characterization of varieties of grapes is particularly useful.

2. To investigate the genome varieties of grapes, has a special importance when harvest plant material. As the leaves are young, quantity and purity dezoxiribonucleic acid (DNA) recorded lower. In If the 12 species investigated, differences were found both the quantity and purity.

3. Right, the regression equation and coefficient of correlation between DNA quantity and purity of DNA are directly dependent on the variety of grapes and extraction method used.

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