

RESULTS CONCERNING THE ANALYSIS OF THE REACTION PRODUCTS RESULTING FROM GENOMIC DNA AMPLIFICATION USING AGAROSE GEL ELECTROPHORESIS FOR POTATOES STUDIED OLD VARIETIES

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Abstract: The author is currently involved in collecting, making an inventory, evaluation and preservation the old varieties from the Western part of Romania. In this paper 8 potato old varieties collected during 20 years and 2 varieties from National Institute of Research and Development for potato and Sugar Beet Brasov are presented. The preservation was carried out in vivo and in vitro. Important changes were observed during this time. In our work we identified many gaps in the knowledge and understanding of the origin of transformations. We made a comparison between two big areas of potato growth: Apuseni Mountains [5] and the Maramures County [3]. In these areas the potato represents the main food in winter. This work opens opportunities for future researches in the field of political and ethical decisions for potato gene pool conservation. Soon the exchange of genetic resources will be a diplomatic issue.

Keywords: potato, varieties, in vitro, molecular evaluation

INTRODUCTION

In plant breeding programs, the old varieties frequently represent an important and valuable gene source, these being characterized by homeostasis, many valuable particularities but most of them still insufficiently known.

The most important characteristics of the old varieties relate to tolerance, various biotic, abiotic stress factors and also to quality. For this reason, old varieties (potato, cucumbers, beans, green salad and herbs) are widely cultivated in spite of being less yielding.

Biochemistry and vegetal genetics witnessed many achievements due to modern research techniques, such as electrophoresis using polyacrylamide gel and column chromatography, which provide high resolution potential. The use of such techniques allowed obtaining useful information regarding the heterogeneity for proteins, enzymes and isoenzymes with esterase, peroxidase and catalase action from different vegetal materials. These studies allowed also the establishment of the fundamental base for new genetic knowledge concerning the role and formation processes of proteins and enzymes found in the vegetal organisms.

The research present were the cognition of characteristic biochemical specific which his needles can permit the characterization of the genotypes of potatoes, the taxonomic identification and selection populations with qualitative superior his indexes with proof complaints and harmful.

The main purpose of the present research studies was to find out new information regarding the specific biochemical characteristics which further allowed the characterization of potato genotypes, taxonomical identification and selection of some old varieties with superior qualitative indices or with disease or pest resistance.

MATERIALS AND METHODS

The biological material was represented by 8 old varieties and 2 genotypes with different genetic and ecologic origin (**Table 1**).

Table 1. Material used to perform the experiments

No.	Provenance locality	Provenance zone
1.	Galbina, 51	Apuseni
2.	Gradistea, 45	Apuseni
3.	Curechui, 90	Apuseni
4.	Strei – Ohaba, 734	Apuseni
5.	Almasul Mare de Munte, 49	Apuseni
6.	Repedea, 100	Maramures
7.	Bistra, 249	Maramures
8.	Salistea de Sus, 909	Maramures
9.	Christian variety	I.C.D.C.S.Z. – Brasov
10.	Desirée variety	I.C.D.C.S.Z. – Brasov

The statistical calculations were used for analysis of the individual variance, the values with central tendency and their deviation ($\bar{x} \pm s_x$) as well as the differences and their deviation ($\bar{d} \pm s_d$) being assessed.

For multiple comparisons we have used specific methods for single factor analysis.

DNA extraction from potato old varieties was performed for all working variants after CTAB method [4].

RESULTS AND DISCUSSIONS

The unilateral or combined in a multiple analysis pointed out the differences among the old varieties with the highest yield (**Fig. 1**).

The comparison of tubers weight from *in situ* growth Apuseni Mountains and Maramures County old varieties (**A** graduation; 2001) with *ex situ* growing in Timisoara conditions (**B** graduation; 2002 and 2003) pointed out relevant results.

The origin of evolution with its environmental conditions represented a valuable interface each year. The Apuseni Mountains old varieties (32) showed a higher capacity of tuber formation. The 28.7%

differences between the two old varieties originating from the Apuseni Mountains and the Maramures County were high and significant: i.e. 86.3 g and 61.5 g respectively.

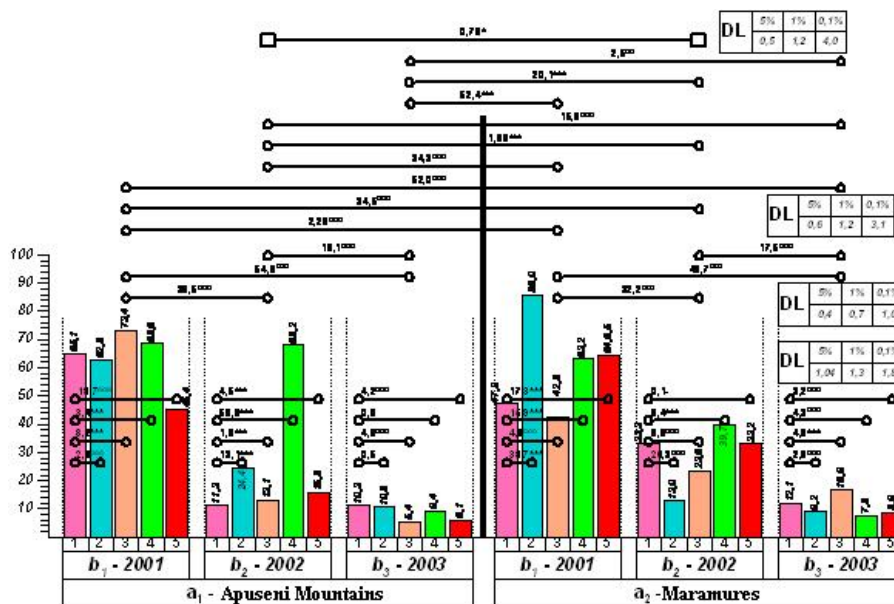


Figure 1. The tuber weight (g) in different old varieties revealing in situ the best yield

The *ex situ* cultivation and evaluation pointed out a considerable decrease of tuber weight. In comparison to 166.7± 11.09 g the general *in situ* average, the potato weight dropped to 27.15 g the general *ex situ* average (2002 and 2003) representing 83.7% decrease.

In the first year of *ex situ* growing the diminishing of tuber weight was high (47.7%) and it was highest (65.0%) in the second year (2002 against 2003).

The *in situ* hierarchy of old varieties was not observed in *ex situ* cultivation. The *Curechiu* 90 from Apuseni area was the best in 2001 and in *ex situ* it was on the last position (5 and 6 in 2002 and 2003 respectively). The *Poiana*-6 was situated the second in rank in their native area. In *ex situ* it pointed out a low homeostasis being the third in rank in 2002 and the last one in 2003. The *Big Almas*-209 was the last genotype from this group (*in situ*) but it was the best in 2002 and 2003.

Within the Maramures group a similar behavior was observed (Fig. 1).

The same analysis was applied to the worthiest yielding old varieties (Fig. 2).

Between the two groups of old varieties the differentiation departure was small 2% but it was significant. The general average of the Maramures old varieties was higher, 33.4 g against Apuseni old varieties 32.79 g.

For *in situ* growing (2001) the Apuseni Mountain old varieties pointed out a higher tuber weight. The differences between the two groups were small but significant (p 1% = 3.5%).

As in the best yielding old varieties the same process of decrease from *in situ* to *ex situ* cultivation was observed (Fig. 2).

The same process of decrease in all biological processes took place in low yielding old varieties too. The tuber weight decline was higher in Apuseni Mountain old varieties 72.4% in 2002 and 86.7% in 2003. The decline was from 63.4 g to 17.5 g and from 63.4 g to 8.4 g in 2002 and 2003 *ex situ* cultivation respectively.

The Maramures old varieties pointed out a better homeostasis. The decrease varied from 67.6% and 82.0% in Timisoara *ex situ* cultivation in 2002 and 2003 respectively.

At all comparisons the differences are significant (p>01%).

The individual old variety analysis the *in situ* hierarchy was not followed in *ex situ* cultivation.

The *Oprisesti*-4 was the best in 2001 in *in situ* cultivation, but it was the last but one in 2002 and the last one in 2003.

10 RAPD primers, selected at random were used for the molecular characterization of the genotypes. One type of DNA was amplified with all of these primers and the best one 5'TGCCGAGCTG3', which generated the highest number of fragments, was used further (Fig. 3).

Agarose gel electrophoresis of the DNA amplification products obtained with 5'TGCCGAGCTG3'.

The selected primer was used to amplify eight the DNA of old varieties and two potato genotypes.

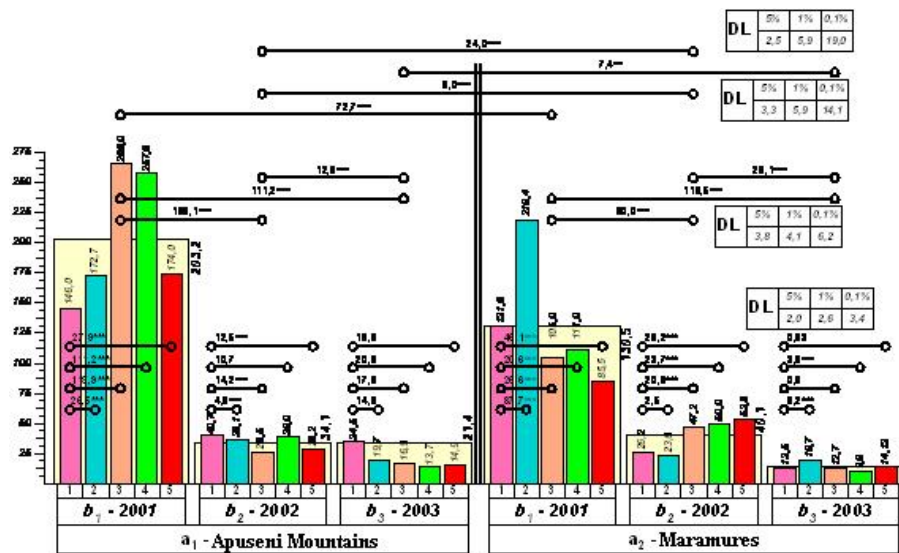


Figure 2. The tuber weight (g) in different old varieties revealing in situ the lowest yield

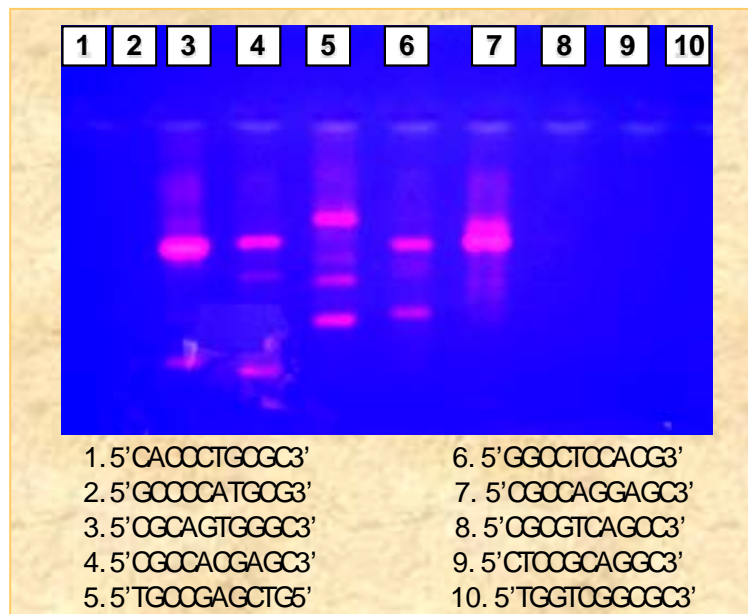


Figure 3. Analysis using polyacrylamide gel electrophoresis 1.5% of the reaction products resulting from DNA amplification with the primer set RAPD – PCR 480 (80% GC)



Figure 4. Analysis using agarose gel electrophoresis (1.5%) of the reaction products formed from genomic DNA amplification using 5'TGCCGAGCTG3' primer

The analysis of amplification fragments has indicated a high polymorphism rate between the studied varieties (Fig. 4).

Most of the fragments were generated for *Bacaia* 18, *Repedea* 100 and *Salistea de Sus* 1600 old varieties.

In case of *Galbina* 51 old variety and *Desirée* variety, the analysis of the amplification fragments revealed the presence of two fragments of extremely low intensity and low molecular weight. The RNA signaling was also slim.

The molecular fingerprinting for *Almasul Mic de Munte* 49 old varieties was different comparing the rest of the studied varieties, 3 low molecular weight fragments being observed. One of the three fragments showed medium intensity and RNA signaling was intense.

In case of *Curechiu* 90 and *Bistra* 249 old varieties, the amplification did not occur because the RAPD primer could not find the complementary sequences in the genome.

Taking in consideration that molecular fingerprint was not correlated with potato pith color, we may regard that genes responsible for this trait are not found in the regions recognized by RAPD primer we have been using.

The low molecular weight smears were common for all studied genotypes. *Bacaia* 18, *Strei-Ohaba* and *Salistea de Sus* 1600 were more intense while in case of *Galbina* 51, *Almasul Mic de Munte* 49 and *Repedea* 100 old varieties, these were weaker.

CONCLUSIONS

- The 5'TGCCGAGCTG3' primer emphasized the polymorphism existing among the old varieties.
- The DNA fingerprint revealed the authenticity of the old varieties analyzed.
- There is not a correlation between the DNA fingerprint and the tuber weight.
- The RNA signal was correlated with the tuber weight character.

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